Electronically signed by: Steven Gallagher (FCCLABS\sgallagher)
Date: Friday, January 04, 2013 12:36:40 PM (GMT -05:00)
Location: FCC
Reason: 02. Case-related document upload

REQUEST FOR LABORATORY SERVICES

DISTRIBUTION:
Original - SRL/LAB
Copy II - Investigative File
Copy III - Office Suspense File

TO:	LABORATORY DIRECTOR	CASE #
LAB NAME	R.D. Satzger Forensic Chemistry Center	2012-BOM-715-0599
LAB ADDRESS	6751 Steger Drive	CASE TITLE
	Cincinnati, OH 45237-3097	Ameridose, LLC

REQUESTING OFFICE				
DATE	MM	DD	YY	OFFICE PHONE
	01	02	2013	(781) 224-4848

SA LAST NAME	SA FIRST NAME	OFFICE
Ridgley	Joseph	Boston

OFFICE ADDRESS	IF EVIDENCE HAS BEEN PREVIOUSLY SUBMITTED, LIST CIE NUMBERS BELOW
FDA-OCI BOM	#174754
401 Edgewater Place, Suite 530	
Wakefield, MA 01880	
SAIC SIGNATURE	Mark Dragonetti Christopher Diesel

CASE SUMMARY

State the problem to be resolved by analysis

Explanations should speak to the following points:

1. Include circumstances/reasons for analysis; state when results are needed by;
2. Case background relative to the request;
3. Comments on priority of analysis;
4. Other lab analysis requested or completed on this or similar evidence.

The items on CIE 174788 were obtained via a search warrant, and they are believed to contain the drug Methylprednisolone Acetate. It is suspected that these items may contain a mold or fungus. It is requested that the items be tested for mold, fungus and other microbiological contaminants.

Item 1 on CIE #174782 represents samples taken from equipment which were also obtained during a search warrant. It is requested that the items be tested for mold, fungus and other microbiological contaminants. Items 3 through 9 are believed to contain the drug Methylprednisolone Acetate. It is also requested that the items be tested for mold, fungus and other microbiological contaminants.

It is requested that the analysis of these items be completed within 30 days.

C.I.E. SERIAL	ITEM #
#174788	1-2
#174782	1, 3-9

OCI CERTIFIED INVENTORY OF EVIDENCE

1. SERIAL NUMBER 174782

2. EVIDENCE OBTAINED FROM
New England Compounding Center
97 Waverly Street
Framingham, MA01970
via search warrant

3. EVIDENCE INVENTORIED BY
Ben Celso
Special Agent

4. SPECIAL AGENT
SIGNATURE Ben Celso
Ben Celso, Special Agent
DATE 12-11-12

5. WITNESS
SIGNATURE Joseph Ridgley
Joseph Ridgley, Special Agent
DATE 12-11-12

6. OCI CASE NUMBER
2012-BOM-715-0599

7. TITLE
Ameridose, LLC

8. OTHER AGENCY NUMBER

9. OCI FIELD OFFICE
Boston Resident Office 789348

10. DATE OF INVENTORY
12/11/2012

11. PAGE 1 OF 1 PAGES

12. OCI REVIEWING SUPERVISOR
SIGNATURE Christopher
DATE 12/12/12

ITEM NO.	DATE RECEIVED	QUANTITY	DESCRIPTION OF EVIDENCE	ESTIMATED VALUE (Rounded To Nearest Dollar)
1	10/31/2012	2	samples, blue/green in color, taken from Flow Mod #FS10300M, Ser. #05-M-17-15-60-90 LF (Control #36)	
2	10/31/2012	1	vial labeled, in part, "Methylpred. AC (PF) 40MG/ML Inject., Lot #08132012@38, necc, 5ML" (Control #28)	
3	10/31/2012	2	containers labeled, in part, "Methylprednisolone Acetate, USP (Micronized), 500g, Lot: 83950/B" (Control #19)	
4	10/31/2012	1	container labeled, in part, "Methylpred. AC (PF) 40MG/ML Inject, Lot #08312012@5, sample" (Control #19)	
5	10/31/2012	4	containers labeled, in part, "Methylpred. AC (PF) 80MG/ML Inject, Lot #09052012@7, sample" (Control #19)	
6	10/31/2012	1	container labeled, in part, "Methylprednisolone AC 80MG/ML Inj, Lot #07302012@72, sample, Preserved" (Control #19)	
7	10/31/2012	6	containers labeled, in part, "Methylprednisolone AC 80MG/ML Inj, Lot #09112012@75, sample, Preserved" (Control #19)	
8	10/31/2012	1	container labeled, in part, "Methylprednisolone AC 40MG/ML Inj, Lot #08082012@103, sample, Preserved" (Control #19)	
9	10/31/2012	2	containers labeled, in part, "Methylpred. AC (PF) 80MG/ML Inject, Lot #09242012@83, sample" (Control #19)	
			TOTAL	

FORM FDA 3400 (2/06)

PART III – CHAIN OF CUSTODY LOG REMAINS WITH EVIDENCE AT ALL TIMES

CHAIN OF CUSTODY LOG

789348

If this inventory consists of more than one page, document the Chain of Custody Log on the reverse of the **FINAL CONTINUATION SHEET.**

ITEM NO.	DATE	ACTION	REGISTRY NUMBER(S)	SIGNATURE
1, 3-9	12/20/12	TOT SA Cl[illegible]	—	
1, 3-9	12-20-12	Received fm EC, Sent to FCC	1ZA5718V0196076011 1ZA5718V0197482624 1ZA5718V0197135642 1ZA5718V0195183835	
Items 1 + 3-9	12/26/12	RECEIVEd at (FCC) for analysis via UPS/TRK #'s	1ZA5718V0196076011 1ZA5718V0197482624 1ZA5718V0195183835 1ZA5718V0197135642	

FORM FDA 3400 (BACK)
(2/06)

Gallagher, Steven

From: Brueggemeyer, Thomas W
Sent: Monday, December 31, 2012 3:04 PM
To: Celso, Benedict
Cc: Gallagher, Steven; Riley, Robert D; Craft, David; Brzezinski, Jennifer; Korchinski, Katie; Woodward, Philip; Flurer, Cheryl L
Subject: RE: Ameridose samples

Hi Ben,

Not sure when you will be back at work, but when you get a chance, could you get us a Request for Laboratory Services form so we can finish up the paperwork processing on these samples?

Thanks.

Have a good New Year.

Tom

From: Brueggemeyer, Thomas W
Sent: Wednesday, December 26, 2012 4:12 PM
To: Celso, Benedict (Benedict.Celso@oci.fda.gov)
Cc: Gallagher, Steven; Riley, Robert D; Craft, David; Brzezinski, Jennifer; Korchinski, Katie; Woodward, Philip; Flurer, Cheryl L
Subject: Ameridose samples

Hi Ben,

Hope you had a nice Christmas. Today we received four boxes under two CIE's for the Ameridose case. We received the CIE paperwork, but not any Request for Laboratory Services forms. Could you send us this form so we can finish the processing of the sample?

I'm off tomorrow and back Friday. You can e-mail the Request paperwork to Steve Gallagher, our sample custodian (I've copied him on this e-mail).

Thanks Ben.

Tom

Thomas W. Brueggemeyer, Ph.D.
Supervisory Chemist
Spectroscopy, Separations and Molecular Biology Section (SSMBS)

1/4/2013 SG 789348

Forensic Chemistry Center (FCC)
U.S. Food & Drug Administration
6751 Steger Dr.
Cincinnati, OH 45237

Office: (513) 679-2700 x2186
Cell: (513) 304-0372

Fax: (513) 679-2761

Gallagher, Steven

From: Brueggemeyer, Thomas W
Sent: Thursday, January 03, 2013 10:27 AM
To: ORA FCC Sample Custodians
Cc: Craft, David
Subject: FW: Lab Request
Attachments: NECC Lab Request 2.pdf

Steve and Phil,

This Request is for the two Ameridose samples which came last week without an OCI Request form. They are the four(?) boxes which have the large bottles in them. Can you finish the paperwork on these? David Craft wants to get started on them.

Thanks.

Tom

From: Ridgley, Joseph [mailto:Joseph.Ridgley@oci.fda.gov]
Sent: Wednesday, January 02, 2013 4:30 PM
To: Brueggemeyer, Thomas W
Cc: Celso, Benedict
Subject: Lab Request

Tom,

Here is a scanned copy of the original lab request for the items you recently received. I can mail you the original today if you need it. Thanks.

Joseph Ridgley
Special Agent
Food & Drug Administration
Office of Criminal Investigations
401 Edgewater Place, Suite 530
Wakefield, MA 01880
(781) 224-4856- desk
(978) 967-7740-cell
(781) 245-7891 - fax
Joseph.Ridgley@oci.fda.gov

UPS CampusShip: View/Print Label

1. **Ensure there are no other shipping or tracking labels attached to your package.** Select the Print button on the print dialog box that appears. Note: If your browser does not support this function select Print from the File menu to print the label.

2. **Fold the printed sheet containing the label at the line so that the entire shipping label is visible. Place the label on a single side of the package and cover it completely with clear plastic shipping tape. Do not cover any seams or closures on the package with the label.** Place the label in a UPS Shipping Pouch. If you do not have a pouch, affix the folded label using clear plastic shipping tape over the entire label.

3. **GETTING YOUR SHIPMENT TO UPS**

   UPS locations include the UPS Store®, UPS drop boxes, UPS customer centers, authorized retail outlets and UPS drivers.
   Find your closest UPS location at: www.ups.com/dropoff
   Take your package to any location of The UPS Store®, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot® or Staples®) or Authorized Shipping Outlet near you. Items sent via UPS Return Services(SM) (including via Ground) are also accepted at Drop Boxes. To find the location nearest you, please visit the Resources area of CampusShip and select UPS Locations.

   Customers with a Daily Pickup
   Your driver will pickup your shipment(s) as usual.

FOLD HERE

BEN.CELSO
781-224-4851
FDA-ORA-HQ-OCI-RO-BORO1
401 EDGEWATER PLACE
WAKEFIELD MA 01880

40 LBS 4 OF 4

SHIP TO:
FORENSIC CHEMISTRY CENTER
U.S. FOOD & DRUG ADMINISTRATION
6751 STEGER DRIVE
CINCINNATI OH 45237-3097

OH 451 9-02

UPS NEXT DAY AIR 1

TRACKING #: 1Z A57 18V 01 9713 5642

BILLING: P/P

CENTER: ORA HQ
Center/Office: oci

CS 14.5.29. WNTIE80 33.0A 10/2012

1/4/2013 SG 789348

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   Find your closest UPS location at: www.ups.com/dropoff

   Take your package to any location of The UPS Store®, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot® or Staples®) or Authorized Shipping Outlet near you. Items sent via UPS Return Services(SM) (including via Ground) are also accepted at Drop Boxes. To find the location nearest you, please visit the Resources area of CampusShip and select UPS Locations.

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FOLD HERE

BEN CELSO
781-224-4851
FDA-ORA-HQ-OCI-RO-BORO1
401 EDGEWATER PLACE
WAKEFIELD MA 01880

40 LBS 3 OF 4

SHIP TO:
FORENSIC CHEMISTRY CENTER
U.S. FOOD & DRUG ADMINISTRATION
6751 STEGER DRIVE
CINCINNATI OH 45237-3097

OH 451 9-02

UPS NEXT DAY AIR 1

TRACKING #: 1Z A57 18V 01 9518 3835

BILLING: P/P

CENTER: ORA HQ
Center/Office: oci

CS 14.5.29. WNTIE80 33.0A 10/2012

UPS CampusShip: View/Print Label

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   Customers with a Daily Pickup
   Your driver will pickup your shipment(s) as usual.

FOLD HERE

BEN CELSO
781-224-4851
FDA-ORA-HQ-OCI-RO-BORO1
401 EDGEWATER PLACE
WAKEFIELD MA 01880

40 LBS 2 OF 4

SHIP TO:
FORENSIC CHEMISTRY CENTER
U.S. FOOD & DRUG ADMINISTRATION
6751 STEGER DRIVE
CINCINNATI OH 45237-3097

OH 451 9-02

UPS NEXT DAY AIR 1
TRACKING #: 1Z A57 18V 01 9748 2624

BILLING: P/P

CENTER: ORA HQ
Center/Office: oci

CS 14.5.29. WNTIE80 33.0A 10/2012

UPS CampusShip: View/Print Label

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FOLD HERE

BEN.CELSO
781-224-4851
FDA-ORA-HQ-OCI-RO-BORO1
401 EDGEWATER PLACE
WAKEFIELD MA 01880

40 LBS 1 OF 4

SHIP TO:
FORENSIC CHEMISTRY CENTER
U.S. FOOD & DRUG ADMINISTRATION
6751 STEGER DRIVE
CINCINNATI OH 45237-3097

OH 451 9-02

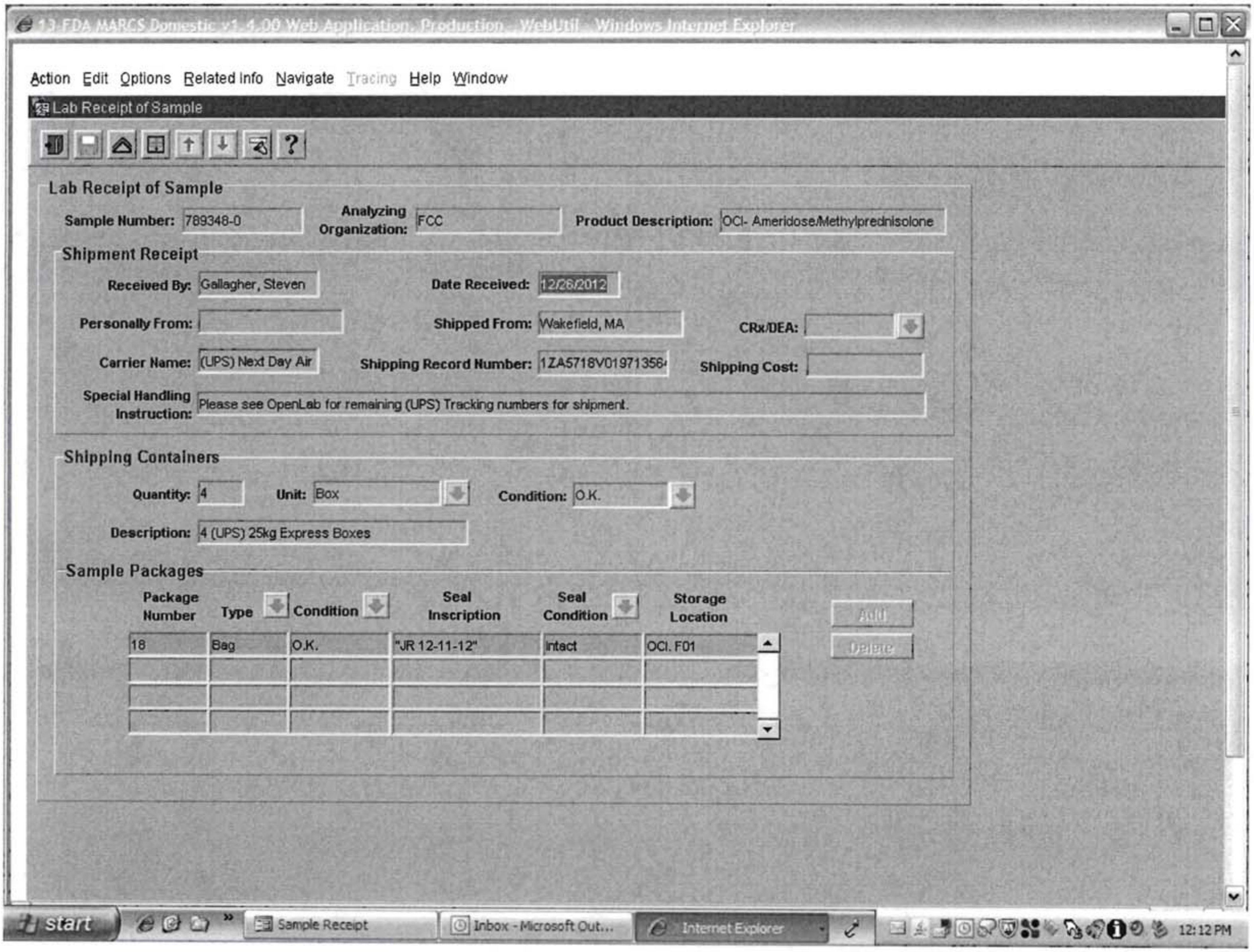
UPS NEXT DAY AIR 1

TRACKING #: 1Z A57 18V 01 9607 6011

BILLING: P/P

CS 14.5.29. WNTIE80 33.0A 10/2012

CENTER: ORA HQ
Center/Office: oci


13 FDA MARCS Domestic v1.4.00 Web Application: Production - WebUtil - Windows Internet Explorer
Action Edit Options Related Info Navigate Tracing Help Window
Lab Receipt of Sample
Add
Delete
start
Sample Receipt
Inbox - Microsoft Out...
Internet Explorer
12:12 PM

Lab Receipt of Sample

Sample Number: 789348-0
Analyzing Organization: FCC
Product Description: OCI- Ameridose/Methylprednisolone

Shipment Receipt

Received By: Gallagher, Steven
Date Received: 12/26/2012
Personally From:
Shipped From: Wakefield, MA
CRx/DEA:
Carrier Name: (UPS) Next Day Air
Shipping Record Number: 1ZA5718V01971356
Shipping Cost:
Special Handling Instruction: Please see OpenLab for remaining (UPS) Tracking numbers for shipment.

Shipping Containers

Quantity: 4
Unit: Box
Condition: O.K.
Description: 4 (UPS) 25kg Express Boxes

Sample Packages

Package Number	Type	Condition	Seal Inscription	Seal Condition	Storage Location
18	Bag	O.K.	"JR 12-11-12"	Intact	OCI. F01

Friday, Jan 04, 2013 12:12 PM

1/4/2013 SG 789348

U.S. Food and Drug Administration, Forensic Chemistry Center
6751 Steger Drive, Cincinnati, Ohio 45237-3097
Telephone: (513) 679-2700 FAX: (513) 679-2761

Case/Sample Summary Report

Date: July 3, 2013

From: David L. Craft, Ph.D., Microbiologist, Organic Branch

Subject: Supplemental Results of Analysis, OCI Case #2012-BOM-715-0599, CIEs #174782 (FDA FACTS #789348), #174730 (FDA FACTS #795706), #174701 (FDA FACTS #795619) and #174792 (FDA FACTS #795675).

To: Ben Celso, Special Agent
Boston Resident Office, OCI/FDA

Through: Cheryl L. Flurer, Ph.D., Director, Organic Branch
Forensic Chemistry Center

Supplemental Summary

Case summaries including sample description and laboratory results for CIEs 174782 (May 8, 2013), 174730 (June 3, 2013), 174701 (June 3, 2013) and 174792 (June 3, 2013) were sent previously as indicated.

The identities of several fungi were determined by the Centers for Disease Control (CDC). The CIE number, the corresponding item number and fungus are shown below.

CIE	Item	Fungal identity
174782	5B	*Sporotrichum pruinosum*
174782	5C	*Sporotrichum pruinosum*
174730	3A	*Exserohilum rostratum*
174730	3B	*Exserohilum rostratum*
174730	3C	*Exserohilum rostratum*
174730	3D	*Exserohilum rostratum*
174730	3E	*Exserohilum rostratum*
174701	16A	*Exserohilum rostratum*
174701	16B	*Exserohilum rostratum*
174701	16D	*Exserohilum rostratum*
174792	15A2	*Aspergillus sydowii*

David L. Craft, Ph.D.

Thomas W. Brueggemeyer, Ph.D.
Reviewer

Centers for Disease Control & Prevention
Fungal Identification Laboratory
Mailstop G11, 1600 Clifton Rd NE, Atlanta, GA 30329, United States

Report Status: Final

Specific Product Name: **None Provided**
Brand/Manufacturer: Lot Number: Expiration Date:

Public Health / International Submitter:

Food and Drug Administration
Cincinnati, Ohio 45237
United States

Sample ID:
Alt. Sample ID:
Specimen ID:
Alt. Specimen ID:

Original Submitter:

Food and Drug Administration
Cincinnati, Ohio 45237
United States

Sample ID:
Alt. Sample ID:
Specimen ID:
Alt. Specimen ID:

Intermediate Submitter:

Katie Korchinski
U.S. FDA Forensic Chemistry
6751 Steger Drive
Cincinnati, Ohio 45237
United States

Email: katie.korchinski@fda.hhs.gov

Sample ID: **789348 Item 5A**
Alt. Sample ID:
Specimen ID:
Alt. Specimen ID:

CDC Specimen ID: **2013008037** CDC Unique ID: **N8K6WFO1**

Material submitted: **Isolate**
Specimen Source: **Commercial Products (usually drugs)**
Specimen source site:
Test order name: **Fungal Identification**

Specimen collected Date: **12/11/2012**
Date sent to CDC:
Date Received: **2/28/2013**
Date Reported: **3/26/2013**

Revision History: No previous reports.

Specific Product Name: **None Provided**

Brand/Manufacturer: Lot Number: Expiration Date:

Public Health / International Submitter IDs

Sample ID: Alt. Sample ID:

Specimen ID: Alt. Specimen ID:

CDC Specimen ID: **2013008037** CDC Unique ID: **N8K6WFO1**

Type of Specimen: **Special Study**

Mold Species Identified: **Basidiomycete fungus**

Test	Result
Colony Texture	**Floccose / Powdery**
Conidiogenous Cell	**Arthric**
Microscopic morphology - mold	**Mycelium Septate / Conidiating**
Thermotolerance	**Growth at 25C / Growth at 37C**
Colony Color Mold	**White**
Cycloheximide Sensitivity	**No Growth**
Benomyl sensitivity	**Growth**
Comments	**This isolate was identified using DNA sequencing. This test has not been cleared or approved by the FDA. It was developed and its performance characteristics were determined by the Mycotic Diseases Branch Reference Lab.**

Comments:
Basidiomycete fungus, most closely resembles Phanerochaete sp.

Approved by: Mary E. Brandt, Ph.D.
Ph: 404-639-0281
Fax: 404-639-3021
E-mail: MBrandt@cdc.gov

Electronically signed by: David L. Craft (FCCLABS \dcraft)
Date: Monday, May 06, 2013 4:13:38 PM (GMT -04:00)
Location: FCC
Reason: 02. Case-related document upload

Electronically signed by: Steven Gallagher (FCCLABS\sgallagher)
Date: Friday, June 28, 2013 1:25:12 PM (GMT -04:00)
Location: FCC
Reason: 02. Case-related document upload

REQUEST FOR LABORATORY SERVICES

DISTRIBUTION:
Original - SRL/LAB
Copy II - Investigative File
Copy III - Office Suspense File

TO: LABORATORY DIRECTOR		CASE #
LAB NAME	R.D. Satzger Forensic Chemistry Center	2012-BOM-715-0599
LAB ADDRESS	6751 Steger Drive	CASE TITLE
	Cincinnati, OH 45237-3097	Ameridose, LLC

REQUESTING OFFICE

DATE	MM	DD	YY	OFFICE PHONE (781) 224-4848
	06	26	2013	

SA LAST NAME	SA FIRST NAME	OFFICE
Celso	Ben	Boston

OFFICE ADDRESS	IF EVIDENCE HAS BEEN PREVIOUSLY SUBMITTED, LIST CIE NUMBERS BELOW
FDA-OCI BOM 401 Edgewater Place, Suite 530 Wakefield, MA 01880	#174754, #174788. #17482, #174701, #174729, #174730, #174786, #174792, #174337, #174338
SAIC SIGNATURE	Mark Dragonetti Christopher Friesel

CASE SUMMARY

State the problem to be resolved by analysis

Explanations should speak to the following points:

1. Include circumstances/reasons for analysis; state when results are needed by;
2. Case background relative to the request;
3. Comments on priority of analysis;
4. Other lab analysis requested or completed on this or similar evidence.

The items on CIE 174701 and 174782 were obtained via search warrant. An analysis is requested to identify the ingredients in Item 78 of CIE 174701. It is suspected that Item 78 contains Methylprednisolone acetate.

In addition it is requested that a volume measurement be conducted for Item 5 of CIE #174782.

It is requested that the analysis of these items be completed within 30 days.

C.I.E. SERIAL	ITEM #

6/28/2013 SG 789348

#174782 #174701	5 78

UPS CampusShip: View/Print Label

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3. **GETTING YOUR SHIPMENT TO UPS**
   UPS locations include the UPS Store®, UPS drop boxes, UPS customer centers, authorized retail outlets and UPS drivers.
   Schedule a same day or future day Pickup to have a UPS driver pickup all your CampusShip packages.
   Hand the package to any UPS driver in your area.
   Take your package to any location of The UPS Store®, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot® or Staples®) or Authorized Shipping Outlet near you. Items sent via UPS Return Services(SM) (including via Ground) are also accepted at Drop Boxes. To find the location nearest you, please visit the Resources area of CampusShip and select UPS Locations.

   Customers with a Daily Pickup
   Your driver will pickup your shipment(s) as usual.

FOLD HERE

BEN.CELSO 5 LBS 1 OF 1
781-224-4851
FDA-ORA-HQ-OCI-RO-BORO1
401 EDGEWATER PLACE
WAKEFIELD MA 01880

SHIP TO:
THOMAS BRUEGGEMEYER, PH.D.
FORENSIC CHEMISTRY CENTER (FCC)
6751 STEGER DRIVE
U.S. FOOD & DRUG ADMINISTRATION
CINCINNATI OH 45237-3097

OH 451 9-02

UPS NEXT DAY AIR 1

TRACKING #: 1Z A57 18V 01 9533 5699

BILLING: P/P

CENTER: ORA HQ
Center/Office: oci

CS 15.1.10. WNTIE80 39.0A 04/2013

Lab Request for
Additional Analysis
Item#5 CI#174782 FACTS# 789348
(RECEIVED 6/28/13 (SG))

6/28/2013 SG 789348

FCC Section Results Sheet

Sample No. 789348

TITLE: Microbiology: USP 61: Examination of Nonsterile Products

PURPOSE: The purpose of the test is to determine the microbial load in the sample.

RESULTS (SUMMARY)
The sample consisted of FACTS #789161, Items #1A, 1B and 2; and FACTS #789348, Item 3A

A portion of each sample unit below was diluted in trypticase soy broth and plated to both trypticase soy agar and Sabouraud dextrose agar to assess the bioburden of the sample.

Sample #/ Item #	Product
789161/ Item 1A	Methylprednisolone Acetate Lot #86959/B
789161/ Item 1B	Methylprednisolone Acetate Lot #86959/B
789161/ Item 2	Methylprednisolone Acetate Lot #85176/G
789348/ Item 3A	Methylprednisolone Acetate Lot #83950/B

No aerobic bacteria or fungi were isolated from the sample contents of any of the Items tested.

See FACTS #789161, Section 524 for analytical results

Analyst:	Checked by:	Report Author:
Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Thursday, January 17, 2013 9:22:15 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS \dcraft) Date: Thursday, January 17, 2013 12:06:04 PM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, May 02, 2013 1:36:45 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above

789348

Ohio Department of Health Laboratory
Microbiology Specimen Submission Form

Ohio Department of Health Laboratory
8995 East Main Street
Building 22
Reynoldsburg, OH 43068

Phone: 888-634-5227
Fax: 614-387-1505
Email: odhlabs@odh.ohio.gov
CLIA Certification # 36D0655844

Note: Fields marked with an asterisk (*) must be completed

Section 1: Patient Information

*Name (Last)
*Name (First)
MI.

Address
City
County
State
Zip

* Sex ○ Male ○ Female ○ Unknown
* Date of Birth (MMDDYYYY)
Chart or Patient ID # 9348-5B
Ethnicity ○ Hispanic/Latino, ○ Non-Hispanic/Non-Latino

Race (check all that apply)
○ White ○ Black ○ American Indian or Alaskan Native ○ Asian ○ Hawaiian native/Pacific Islander ○ Other

Section 2: Submitter Information

* Agency Name: US FDA Forensic Chem
* Contact Name: David Craft
Address: 6751 Steger Dr.
City: Cincinnatti
County:
State: OH
Zip: 45237
* Phone Number: 5136792700
*Fax Number: 5136792761

Section 3: Specimen Information (Check all that apply)

* Specimen
○ Clinical (Human Origin) ○ Isolate (Human Origin)
○ Clinical (Non-human Origin) ☒ Isolate (Non-human Origin)

* Collection Date: 5/23/13
* Onset Date:
ODH Outbreak Number:
Agent Suspected: Fungal

* Specimen Type
○ BAL ○ CSF ○ Urine
○ Serum, Acute ○ Serum, Convalescent ○ Sputum
○ Stool, Broth ○ Stool, Bulk ○ Stool, Cary Blair
○ Plasma ○ Whole Blood
○ Swab, Specify ○ Tissue, Specify
○ Wound, Specify ○ Body Fluid, Specify
☒ Other, Specify IV Medication

Section 4: Test Requested (Check all that apply)

**ODH approval required prior to submission; Contact 614-995-5599

Virology
○ Respiratory virus
○ Other

Mycology & Parasitology
○ *Coccidioides immitis*
○ *Cryptosporidium*
☒ Other: Fungal ID

Mycobacteriology
○ Smear only
○ Culture for AFB
○ Nucleic acid amplification
○ Isolate for Identification
○ Primary susceptibility testing (SM, INH, RIF, EMB, PZA)
○ Genotyping only
○ Other:

Microbiology
○ Pulsed-field gel electrophoresis (PFGE)**
○ Enteric Isolate for ID
○ *Salmonella*
○ *Shigella*
○ *E. coli* O157 (STEC)
○ *E. coli* non-O157 (STEC)
○ *Campylobacter*
○ Bio-threat Agent:
○ *Yersinia*
○ *Neisseria meningitidis*
○ *Haemophilus influenzae*
○ *Listeria monocytogenes*
○ *Clostridium botulinum***
○ Norovirus**
○ Other:

Comments

*****FINAL REPORT*****
SEE ATTACHED CDC FINAL REPORT

For Use by the Ohio Department of Health Laboratory Only

Date Received: 6/24/13
Date Reported: 6/24/13

Billing:
○ Fee Due MI
MI
○ Exemption

13000145

HEA 2530 (ODH Lab Rev 2/2012)

Electronically signed by: Thomas W. Brueggemeyer (FCCLABS\tbrueggemeyer)
Date: Tuesday, July 16, 2013 11:23:58 AM (GMT -04:00)
Location: fcc
Reason: 02. Case-related document upload

789348

Centers for Disease Control & Prevention
Fungal Identification Laboratory
Mailstop G11, 1600 Clifton Rd NE, Atlanta, GA 30329, United States

Report Status: Final

Specific Product Name: **None Provided**
Brand/Manufacturer:
Lot Number:
Expiration Date:

Public Health / International Submitter:

US FDA Forensic Chemistry Center
6751 Steger Dr.
Cincinnati, Ohio 45237
United States

Email: david.craft@fda.hhs.gov

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-5B**
Alt. Specimen ID:

Original Submitter:

Dr David Craft
US FDA Forensic Chemistry Center
6751 Steger Dr.
Cincinnati, Ohio 45237
United States

Email: david.craft@fda.hhs.gov

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-5B**
Alt. Specimen ID:

13000145

CDC Specimen ID: **2013734048**
CDC Unique ID: **N8K6SNRR**

Material submitted: **Isolate**
Specimen Source: **IV Medications**
Specimen source site:
Test order name: **Fungal Identification**

Specimen collected Date:
Date sent to CDC: **5/23/2013**
Date Received: **5/24/2013**
Date Reported: **6/21/2013**

Revision History: No previous reports.

Date Printed: 6/21/2013

CLIA ID # 11D0668319

Centers for Disease Control & Prevention
Fungal Identification Laboratory

Specific Product Name: **None Provided**
Brand/Manufacturer:
Lot Number:
Expiration Date:

Public Health / International Submitter IDs

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-5B**
Alt. Specimen ID:

CDC Specimen ID: **2013734048**
CDC Unique ID: **N8K6SNRR**

Type of Specimen: **Special Study**

Mold Species Identified: **Sporotrichum pruinosum**

Test	Result
Colony Texture	**Powdery**
Conidiogenous Cell	**Arthroconidia**
Microscopic morphology - mold	**Mycelium Septate / Conidiating**
Thermotolerance	**Growth at 25C, 37C, and 40C**
Cycloheximide Sensitivity	**No Growth**
Benomyl sensitivity	**Growth**
Comments	**This isolate was identified using DNA sequencing. This test has not been cleared or approved by the FDA. It was developed and its performance characteristics were determined by the Mycotic Diseases Branch Reference Lab.**

Approved by:
Mary E. Brandt, Ph.D.
Ph: 404-639-0281
Fax: 404-639-3021
E-mail: MBrandt@cdc.gov

13000145

Revision History: No previous reports.

Date Printed: 6/21/2013

CLIA ID # 11D0668319

789348

Ohio Department of Health Laboratory
Microbiology Specimen Submission Form

Ohio Department of Health Laboratory
8995 East Main Street
Building 22
Reynoldsburg, OH 43068

Phone: 888-634-5227
Fax: 614-387-1505
Email: odhlabs@odh.ohio.gov
CLIA Certification # 36D0655844

Note: Fields marked with an asterisk (*) must be completed

Section 1: Patient Information

*Name (Last)		*Name (First)			MI.
Address		City	County	State	Zip
* Sex ○ Male ○ Female ○ Unknown	* Date of Birth (MMDDYYYY)	Chart or Patient ID # 9348-SC	Ethnicity ○ Hispanic/Latino, ○ Non-Hispanic/Non-Latino		

Race (check all that apply)
○ White ○ Black ○ American Indian or Alaskan Native ○ Asian ○ Hawaiian native/Pacific Islander ○ Other

Section 2: Submitter Information

* Agency Name US FDA Forensic Chem		* Contact Name David Craft		Address 6751 Steger Dr.	
City Cincinnatti	County	State OH	Zip 45237	* Phone Number 5136792700	*Fax Number 5136792761

Section 3: Specimen Information (Check all that apply)

* Specimen
○ Clinical (Human Origin) ○ Isolate (Human Origin)
○ Clinical (Non-human Origin) ☒ Isolate (Non-human Origin)

* Collection Date: 5/23/13
* Onset Date:

ODH Outbreak Number:
Agent Suspected: Fungal

* Specimen Type
○ BAL ○ CSF ○ Urine
○ Serum, Acute ○ Serum, Convalescent ○ Sputum
○ Stool, Broth ○ Stool, Bulk ○ Stool, Cary Blair
○ Plasma ○ Whole Blood
○ Swab, Specify ____ ○ Tissue, Specify ____
○ Wound, Specify ____ ○ Body Fluid, Specify ____
☒ Other, Specify IV Medication

Section 4: Test Requested (Check all that apply)

**ODH approval required prior to submission; Contact 614-995-5599

Virology
○ Respiratory virus
○ Other ____

Mycology & Parasitology
○ *Coccidioides immitis*
○ *Cryptosporidium*
☒ Other: Fungal ID

Mycobacteriology
○ Smear only
○ Culture for AFB
○ Nucleic acid amplification
○ Isolate for Identification
○ Primary susceptibility testing (SM, INH, RIF, EMB, PZA)
○ Genotyping only
○ Other:

Microbiology
○ Pulsed-field gel electrophoresis (PFGE)**
○ Enteric Isolate for ID
○ *Salmonella*
○ *Shigella*
○ *E. coli* O157 (STEC)
○ *E. coli* non-O157 (STEC)
○ *Campylobacter*
○ Bio-threat Agent:
○ *Yersinia*
○ *Neisseria meningitidis*
○ *Haemophilus influenzae*
○ *Listeria monocytogenes*
○ *Clostridium botulinum***
○ Norovirus**
○ Other:

Comments

*****FINAL REPORT*****
SEE ATTACHED CDC FINAL REPORT

For Use by the Ohio Department of Health Laboratory Only

Date Received: 6/24/13
Date Reported: 6/24/13

Billing:
○ Fee Due MI ____
MI ____
○ Exemption ____

13000146

HEA 2530 (ODH Lab Rev 2/2012)

789348

Centers for Disease Control & Prevention
Fungal Identification Laboratory
Mailstop G11, 1600 Clifton Rd NE, Atlanta, GA 30329, United States

Report Status: Final

Specific Product Name: **None Provided**
Brand/Manufacturer: Lot Number: Expiration Date:

Public Health / International Submitter:

US FDA Forensic Chemistry Center
6751 Steger Dr.
Cincinnati, Ohio 45237
United States

Email: david.craft@fda.hhs.gov

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-SC**
Alt. Specimen ID:

Original Submitter:

Dr David Craft
US FDA Forensic Chemistry Center
6751 Steger Dr.
Cincinnati, Ohio 45237
United States

Email: david.craft@fda.hhs.gov

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-SC**
Alt. Specimen ID:

13000146

CDC Specimen ID: **2013734047** CDC Unique ID: **N8K6SNRL**

Material submitted: **Isolate**
Specimen Source: **IV Medications**
Specimen source site:
Test order name: **Fungal Identification**

Specimen collected Date:
Date sent to CDC: **5/23/2013**
Date Received: **5/24/2013**
Date Reported: **6/21/2013**

Revision History: No previous reports.
Date Printed: 6/21/2013 CLIA ID # 11D0668319

789348

Centers for Disease Control & Prevention
Fungal Identification Laboratory

Specific Product Name: **None Provided**
Brand/Manufacturer: Lot Number: Expiration Date:

Public Health / International Submitter IDs

Sample ID:
Alt. Sample ID:
Specimen ID: **9348-SC**
Alt. Specimen ID:

CDC Specimen ID: **2013734047** CDC Unique ID: **N8K6SNRL**

Type of Specimen: **Special Study**

Mold Species Identified: **Sporotrichum pruinosum**

Test	Result
Colony Texture	**Powdery**
Conidiogenous Cell	**Arthroconidia**
Microscopic morphology - mold	**Mycelium Septate / Conidiating**
Thermotolerance	**Growth at 25C, 37C, and 40C**
Colony Color Mold	**White**
Cycloheximide Sensitivity	**No Growth**
Benomyl sensitivity	**Growth**
Comments	**This isolate was identified using DNA sequencing. This test has not been cleared or approved by the FDA. It was developed and its performance characteristics were determined by the Mycotic Diseases Branch Reference Lab.**

Approved by: Mary E. Brandt, Ph.D.
Ph: 404-639-0281
Fax: 404-639-3021
E-mail: MBrandt@cdc.gov

13000146

Revision History: No previous reports.

Date Printed: 6/21/2013

CLIA ID # 11D0668319

FCC Sample Custody Sheet		Sample No. 789348	
	DESCRIPTION/IDENTIFICATION	**FROM**	**TO**
1	Item 3A	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Tuesday, January 08, 2013 10:34:45 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Tuesday, January 08, 2013 10:35:30 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above
2	Items 4, 5A, 6, 7A, 8 and 9A	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Tuesday, January 08, 2013 11:41:41 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: Katie L. Korchinski (FCCLABS\kkorchinski) Date: Tuesday, January 08, 2013 11:42:21 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above
3	Item 1A and 1B	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Tuesday, January 15, 2013 9:36:26 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Tuesday, January 15, 2013 9:37:01 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above
4	Remainder of item 3A	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Thursday, February 28, 2013 10:49:34 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, February 28, 2013 10:50:06 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above
5	Remainder of items 4, 5A, 6, 7A, 8 and 9A	Electronically signed by: Katie L. Korchinski (FCCLABS\kkorchinski) Date: Thursday, February 28, 2013 10:51:25 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, February 28, 2013 10:52:05 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above
6	Items 5B and 5C	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Wednesday, April 10, 2013 9:05:57 AM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Wednesday, April 10, 2013 9:06:36 AM (GMT -04:00) Location: FCC Reason: 01. Reason given above
7	Remainder of Items 5B and 5C	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Friday, April 26, 2013 2:22:55 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Friday, April 26, 2013 2:24:09 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above
8			
9			
10			
11			
Corrections:		Final Signature: Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, May 02, 2013 11:53:47 AM (GMT -04:00) Location: FCC Reason: 01. Reason given above	

U.S. Food and Drug Administration, Forensic Chemistry Center
6751 Steger Drive, Cincinnati, Ohio 45237-3097
Telephone: (513) 679-2700 FAX: (513) 679-2761

Case/Sample Summary Report

Date: May 8, 2013

From: David L. Craft, Ph.D., Microbiologist, Organic Branch

Subject: Results of Analysis, OCI Case #2012-BOM-715-0599, CIE #174782 (FDA FACTS #789348)

To: Joseph Ridgley, Special Agent
Boston Resident Office, OCI/FDA

Through: Cheryl L. Flurer, Ph.D., Director, Organic Branch Cheryl L Flurer
Forensic Chemistry Center

I. Description of Samples Received for Analysis

Evidence described in part under CIE #174782 was received on December 26, 2012 via UPS and consisted of eight items. Item 1 consisted of two bottles containing a green substance. Two bottles of Item 1 were designated in the laboratory as either Item 1A or 1B. Item 3 consisted of two bottles labeled in part "MEDISCA…METHYLPREDNISOLONE ACETATE, USP (Micronized)…500g…Lot: 83950/B Exp: 01/17" containing a white powder. One bottle of Item 3 was designated in the laboratory as Item 3A. Item 4 consisted of one bottle labeled in part "NEW ENGLAND COMPOUNDING CTR…METHYLPRED. AC (PF)…40MG/ML …Lot#08312012@5". Item 5 consisted of four bottles labeled in part "NEW ENGLAND COMPOUNDING CTR…METHYLPRED. AC (PF)…80MG/ML…Lot#09052012@7". Three bottles of Item 5 were designated in the laboratory as Item 5A, 5B and 5C. Item 6 consisted of one bottle labeled in part "NEW ENGLAND COMPOUNDING CTR…METHYLPREDNISOLONE AC……80MG/ML…… Lot#07302012@72". Item 7 consisted of six bottles labeled in part "NEW ENGLAND COMPOUNDING CTR……METHYLPREDNISOLONE AC…..80MG/ML…..Lot#09112012@75". One bottle of Item 7 was designated in the laboratory as Item 7A. Item 8 consisted of one bottle labeled in part "NEW ENGLAND COMPUNDING CTR……METHYLPREDNISOLONE AC…40MG/ML…..Lot#08082012@103". Item 9 consisted of two bottles labeled in part "NEW ENGLAND COMPOUNDING CTR…METHYLPRED. AC (PF)…40MG/ML…..Lot#09242012@83". One bottle of Item 9 was designated in the laboratory as Item 9A. Items 4, 5, 6, 7, 8 and 9 contained a milky white substance.

II. Analytical Tests Performed on Samples

Microbiological analysis was performed on portions of the contents of Items 1A, 1B, 3A, 4, 5A, 5B, 5C, 6, 7A, 8 and 9A. Fungal identification of isolated fungi was or will be performed by the Centers for Disease Control (CDC). The remaining items were not analyzed.

III. Results and Conclusions

No aerobic bacteria or fungi were isolated from the analyzed portions of Items 1A, 1B, 3A, 4, 6, 7A, 8 or 9A. A fungus, most closely related to the genus *Phanerochaete*, was isolated from a portion of the contents of Item 5A and identified by the CDC. One fungus was isolated each from a portion of the contents of Items 5B and 5C. The identity of these fungi will be performed by the CDC. In addition, a bacterium most closely related to *Bacillus altitudinis* was isolated from a portion of the contents of Item 5C.

IV. Sample Retention/Disposition/Feedback Information

This evidence associated with this sample will be retained by the Forensic Chemistry Center pending instructions from your office for disposition. If you have any questions, concerns or a need for additional information, please do not hesitate to contact me at (513) 679-2700 Ext. 2207, or Thomas W. Brueggemeyer at (513) 679-2700 Ext. 2186.

David L. Craft, Ph.D.

Section Authors Concurrences

523, David L. Craft, Ph.D.____________________

524 and 524a, Jennifer L. Brzezinski, Ph.D.____________________

715, Katie L. Korchinski____________________

Thomas W. Brueggemeyer, Ph.D.
Reviewer

FCC Section Results Sheet

Sample No. 789348

TITLE: Microbiological Analysis

PURPOSE: To determine if fungi are present in the sample contents

RESULTS (SUMMARY)

FCC SOP L007 “Microbiology and PCR Analysis” was used as guidance.

No fungi were isolated from the contents of either Items 1A or 1B.

System controls performed as expected.

Analyst:	Checked by:	Report Author:
Electronically signed by: David L. Craft (FCCLABS \dcraft) Date: Wednesday, February 06, 2013 11:25:10 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Wednesday, February 06, 2013 11:26:28 AM (GMT -05:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, May 02, 2013 1:37:43 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above

FCC Continuation Sheet

Sample No. 789348

Equipment and Reagents

Trypticase Soy Broth (TSB), 10 ml, Remel, $^{\#}$R07226, lot $^{\#}$261484, exp. date 10/17/2013
Malt Extract Agar (MEA), per FDA BAM, prepared locally
Remel Quanti-cult, *Aspergillus brasilliensis* (Ab), ATCC 16404, R4731100, Lot#243449, Exp. Date 3/31/2014

Method

FCC SOP L007 “Microbiology and PCR Analysis” was used as guidance.

1/15/2013

Each item was sampled and plated to MEA, aseptically transferred to 10mL TSB broth and incubated at 25°C. MEA and TSB were processed as uninnoculated media controls.

1/29/2013

No growth observed on any media. A 10µL portion of each TSB culture from each item was plated onto MEA, in duplicate. A Quanti-cult of Ab was used to spike the TSB cultures of Items 1A and 1B individually. The cultures were incubated at 25°C.

2/1/2013

No growth was observed on the MEA plates. Growth was observed in the spiked-TSB cultures of each item.

FCC Section Results Sheet

Sample No. 789348

TITLE: Microbiology

PURPOSE: The purpose of the test is to isolate and identify any aerobic bacteria and/or fungi in the sample.

RESULTS (SUMMARY)

The sample consisted of two large clear plastic bottles each containing a white, opaque liquid (Item 5).

A portion of the contents of each bottle was diluted in trypticase soy broth and monitored for growth.

A mold and a bacterium were isolated from the sample contents. All controls performed as expected.

Based on comparison to the MicroSeq database, the bacterial species isolated from the sample contents was most closely related to *Bacillus altitudinis*.

The mold isolates were sent to The Centers for Disease Control and Prevention (CDC) in Atlanta, GA for identification.

Analyst:	Checked by:	Report Author:
Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Monday, April 29, 2013 12:49:05 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS \dcraft) Date: Monday, April 29, 2013 1:14:34 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, May 02, 2013 1:38:47 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above

Methods:
USP71 (Sterility Tests)-used as guidance following the instructions for Direct Inoculation of the Culture Medium. (aerobic bacteria and fungi) Version date: December 1, 2012
FCC SOP E162 (1.0)-Applied Biosystems 3130xl Genetic Analyzer
FCC SOP E163 (2.0)-Applied Biosystems (AB) MicroSEQ Microbial Identification System
FCC SOP L007 (2.0)-Microbiology and PCR-based Analysis
Fast MicroSeq® 500 16S rDNA Bacterial Identification Kit-Applied Biosystems part #4393012; Rev. B 01/2010
USB® ExoSAP-IT® PCR Product Cleanup Brief Protocol-USB Affymetrix; P-782008; rev 10/10
PERFORMA® DTR Gel Filtration Cartridges Product Summary-EdgeBio v.17

Equipment and reagents;
Trypticase-Soy Broth (TSB) 500 ml-Remel, KS; Cat #R112732; Lot #302904; Exp 2014/1/28
Malt Extract Agar (MEA)-prepared locally
Trypticase Soy Agar (TSA)-B-D, MD; Cat #221283; Lot #3009268; Exp 2013 05 09
Sabouraud Dextrose Agar (SAB)-B-D, MD; Cat #221278 Lot #2325032; Exp 2013 04 26
Trypticase-Soy Agar Slants (TSA)-Remel, KS; Cat #R064862; Lot #248724; Exp 2013/9/18
Sabouraud Dextrose Agar Slants-Remel, KS; Cat #R08722; Lot #252333; Exp 2013/9/26
PrepMan™Ultra-Applied Biosystems; Cat #4318980; Lot #1212145; Exp Date 20JUN13
Nuclease-free water-Ambion; Cat #AM9937; Lot #100306
FAST MicroSeq 500 16S rDNA PCR kit-Applied Biosystems Cat #4370489; Lot #1205078 Exp 14May2013
MicroSeq 500 16S rDNA Sequencing Kit-Applied Biosystems; Cat #4346480; Lot #1205101; Exp 08May2013
Veriti Thermocycler-Applied Biosystems
ExoSAP-IT-USB Corp.; Cat #440829; Lot #4146298; Exp: 7/27/13
Performa DTR Gel Filtration Cartridges-Edge Bio; Cat #98780; Lot #160363; Exp 7/13
HiDi formamide-Applied Biosystems; Cat #4311320; Lot #1103054
Applied Biosystems 3130xl Genetic Analyzer-Serial #21279-017; FDA #5079068
3130 POP-7 Polymer-Applied Biosystems-Cat #4363785; Lot #1212091; Exp 26AUG13
Gram Stain Kit-B-D; MD; Cat #B12539
Gram Check Slides-Fisher, IL; Cat #08-801; Lot #431; Exp 12-13

Sample Prep (789348, Items 5B-5C): 4/9/13

1. Each Item consisted of a plastic bottle containing a white, opaque liquid.
2. 50 ml portions of the sample liquid were added to a 500 ml bottle of TSB (2 portions were taken from each bottle-100 ml sampling total for each Item).
3. An uninoculated bottle of TSB was used as a negative control.
4. The bottles were incubated at 25°C with ambient atmosphere.

4/16/2014

1. Evidence of growth is observed in all of the sample portions.
2. 10 μl of each sample portion is plated to MEA, TSA and SAB, streaking for isolation.
3. The plates were incubated at 25°C with ambient atmosphere.

4/18/13

1. A white mold was isolated from Item 5B.
2. A white mold and a bacterium were isolated from Item 5C.
3. The molds were streaked to duplicate TSA and SAB slants.
4. The bacterium was streaked to a TSA plate.
5. The plates and slants were incubated at 25°C with ambient atmosphere.

4/19/2013
1. The bacterial isolate was Gram-stained.

4/25/2013
1. DNA was extracted from the bacterial isolate and MicroSeq analysis was performed.

Appendix 1- Microbiology Results

Sample # 789348 Analyst/# Brzezinski/524

Method

System controls used as indicated in method: Explain deviation:

Positive [e.g., Growth promotion (Y/N)] Not appli

Negative [e.g.,Uninoculated media (Y/N)] Yes

Other isolates processed simultaneously? Yes If so, list isolate(s): 789348 5C-1; 789348 5C-2

Will PCR be used for this isolate?(Y/N) No If so, attach Appendix 2

List additional isolates simultaneously processed for PCR:

Culture Results

Isolate # 789348 5B-1

1) Date, media, lot #, exp. date, Color, Colonial Morphology, Hemolysis (α, β, γ), Comments

i. 4/19/2013 See page 524-2 for media info: White fuzzy spreading colony on both SAB and MEA

ii. (no growth on TSA)

iii.

2) Gram reaction (slide Lot #, Exp. date, QC OK?)

Positive/Negative/Variable

Morphology:

Form:

3) Additional Tests (i.e., Catalase, Oxidase)

Test/Result

QC organism OK? Positive Negative

Test/Result

QC organism OK? Positive Negative

Test/Result

QC organism OK? Positive Negative

4) Results - Identification by: Attach results

i. Vitek 2

ii. MicroSeq®

iii. PCR

Appendix 1- Microbiology Results

Sample # 789348 Analyst/# Brzezinski/524

Method

System controls used as indicated in method: Explain deviation:

Positive [e.g., Growth promotion (Y/N)] Not appli

Negative [e.g.,Uninoculated media (Y/N)] Yes

Other isolates processed simultaneously? Yes If so, list isolate(s): 789348 5B-1; 789348 5C-2

Will PCR be used for this isolate?(Y/N) No If so, attach Appendix 2

List additional isolates simultaneously processed for PCR:

Culture Results

Isolate # 789348 5C-1

1) Date, media, lot #, exp. date, Color, Colonial Morphology, Hemolysis (α, β, γ), Comments

i. 4/19/2013 See page 524-2 for media info: White fuzzy spreading colony on both SAB and MEA

ii. (no growth on TSA)

iii.

2) Gram reaction (slide Lot #, Exp. date, QC OK?)

Positive/Negative/Variable

Morphology:

Form:

3) Additional Tests (i.e., Catalase, Oxidase)

Test/Result

QC organism OK? Positive Negative

Test/Result

QC organism OK? Positive Negative

Test/Result

QC organism OK? Positive Negative

4) Results - Identification by: Attach results

i. Vitek 2

ii. MicroSeq®

iii. PCR

Appendix 1- Microbiology Results

Sample # 789348 Analyst/# Brzezinski/524

Method
System controls used as indicated in method: Explain deviation:
Positive [e.g., Growth promotion (Y/N)] Not appli
Negative [e.g.,Uninoculated media (Y/N)] Yes
Other isolates processed simultaneously? Yes If so, list isolate(s): 789348 5B-1; 789348 5C-1

Will PCR be used for this isolate?(Y/N) Yes If so, attach Appendix 2
List additional isolates simultaneously processed for PCR: NA

Culture Results
Isolate # 789348 5C-2

1) Date, media, lot #, exp. date, Color, Colonial Morphology, Hemolysis (α, β, γ), Comments
   - i. 4/19/2013-See page 524-2 for media info: Small, circular, dry, tan, slightly irregular colonies on T
   - ii. (very small white colonies on MEA and SAB
   - iii.

2) Gram reaction (slide Lot #, Exp. date, QC OK?) Lot #431; Exp 12-13; QC OK

   Positive/Negative/Variable Positive

   Morphology: Rods small, short rods-no apparent spor

   Form: Clusters

3) Additional Tests (i.e., Catalase, Oxidase)

   Test/Result NA
   QC organism OK? Positive ☐ Negative ☐
   Test/Result
   QC organism OK? Positive ☐ Negative ☐
   Test/Result
   QC organism OK? Positive ☐ Negative ☐

4) Results - Identification by: Attach results

   - i. Vitek 2
   - ii. MicroSeq® Bacillus altitudinis
   - iii. PCR

Appendix 2-PCR Checklist

Sample # 789348
Analyst/# Brzezinski/524

1) Decontamination Procedure

Sample Prep Area
Before Preparation 4/25/2013 JLB (Date/Initials)
Following Preparation 4/25/2013 JLB (Date/Initials)

PCR Assembly
Before Assembly 4/25/2013 JLB (Date/Initials)
Following Assembly 4/25/2013 JLB (Date/Initials)

Equipment Decontamination, if needed
Before Preparation NA (Date/Initials)
Following Preparation NA (Date/Initials)

2) PCR reaction Disposal

4/26/2013 JLB (Date/Initials)(Comments)

Applied Biosystems

Generated at: 26 Apr 2013 at 07:47:25 EDT

Project : 789348_5_26_13 Specimen : Specimen1 Specimen2 Specimen3 Kit : Bacterial500Kit

Specimen	% Match	Sequence Entry	Library
789348	99.95	Bacillus altitudinis (DSM=21631)	AB_Bacterial500Lib_2.2
	99.14	Bacillus pumilus (ATCC=7061)	AB_Bacterial500Lib_2.2
	99.14	Bacillus safensis (DSM=19292)	AB_Bacterial500Lib_2.2
NEG			No Libraries Searched Against
POS	100.00	Escherichia coli (ATCC=11303)	AB_Bacterial500Lib_2.2
	100.00	Escherichia coli W3110 (Sigma=W3110)	AB_Bacterial500Lib_2.2
	99.90	Escherichia coli (ATCC=35382)	AB_Bacterial500Lib_2.2

Project Creator: FDAFCC3130: David Craft
Printed by: FDAFCC3130: David Craft

MicroSeqID v2.1.1 Analysis QC Report

Generated at: 26 Apr 2013 at 07:45:36 EDT

Summary

Project	789348_5_26_13
Project Creation Date	26 Apr 2013 at 07:41:43 EDT
Project Modification Date	26 Apr 2013 at 07:41:53 EDT

Specimens in Report
789348, NEG, POS

Specimen Analysis

Specimen	# Samples	Basecalling	Filter	Assembly	Specimen Score	Top Match	% Match	Consensus Length	Library Entry Length	Comments
789348	2	🟩	🟩	🟩	45	Bacillus altitudinis (DSM=21631)	99.95	496	495	
NEG	2	⚠	🛑	🛑	0					
POS	2	🟩	🟩	🟩	43	Escherichia coli (ATCC=11303)	100.00	490	489	

Successful Analysis 🟩 Quality Alert ⚠ Failed Analysis 🛑

Sample Analysis

Project Creator: FDAFCC3130: David Craft
Printed by: FDAFCC3130

MicroSeqID v2.1.1   Analysis QC Report

Generated at: 26 Apr 2013 at 07:45:36 EDT

Specimen	Sample	Step	Description
NEG	D03_789348_NEGR	Basecalling	Bad Data; Error number = 30331Analysis failed due to poor data quality : (20321)
NEG	C03_789348_NEGF	Filter	Clear range less than minimum allowed (0<50); Sample score less than minimum allowed (0<20)
NEG	D03_789348_NEGR	Filter	Incomplete results presented from previous stage
NEG	-	Assembly	Assembly failed: No samples available to assemble

MicroSeqID v2.1.1 Library Search Report

Applied Biosystems

Generated at: 26 Apr 2013 at 07:44:38 EDT

Summary

Project	789348_5_26_13
Project Creation Date	26 Apr 2013 at 07:41:43 EDT
Project Modification Date	26 Apr 2013 at 07:41:53 EDT

Specimens in Report
789348, NEG, POS

Library

Library	Creation Date	Modification Date	Comments
AB_Bacterial500Lib_2.2	04 Sep 2009 at 09:51:58 EDT	04 Sep 2009 at 09:53:26 EDT	This is a proprietary library provided by Applied Biosystems

Hit List

Specimen	Library	Library Entry Name	% Match	Consensus Length	Library Entry Length	Total Mismatches
789348	AB_Bacterial500Lib_2.2	Bacillus altitudinis (DSM=21631)	99.95	496	495	1
789348	AB_Bacterial500Lib_2.2	Bacillus pumilus (ATCC=7061)	99.14	496	495	5
789348	AB_Bacterial500Lib_2.2	Bacillus safensis (DSM=19292)	99.14	496	495	5
POS	AB_Bacterial500Lib_2.2	Escherichia coli (ATCC=11303)	100.0	490	489	1

MicroSeqID v2.1.1 Library Search Report

Applied Biosystems

Generated at: 26 Apr 2013 at 07:44:38 EDT

Specimen	Library	Library Entry Name	% Match	Consensus Length	Library Entry Length	Total Mismatches
POS	AB_Bacterial500Lib_2.2	Escherichia coli W3110 (Sigma=W3110)	100.0	490	489	1
POS	AB_Bacterial500Lib_2.2	Escherichia coli (ATCC=35382)	99.9	490	489	2

Concise Alignment

789348

Entry Name	244	
789348	R	
Bacillus altitudinis (DSM=21631) 99.95	G	

NEG

Entry Name	Loc	

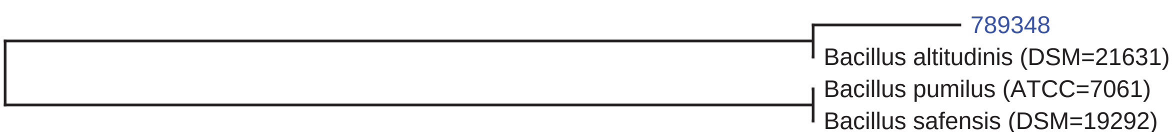
POS

Entry Name	1	
POS	R	
Escherichia coli (ATCC=11303) 100.00	A	

Phylogenetic Tree

Specimen : 789348

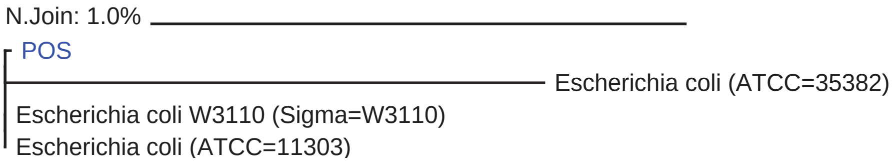
N.Join: 1.0%


789348
Bacillus altitudinis (DSM=21631)
Bacillus pumilus (ATCC=7061)
Bacillus safensis (DSM=19292)

MicroSeqID v2.1.1 Library Search Report

Generated at: 26 Apr 2013 at 07:44:38
EDT

Specimen : POS

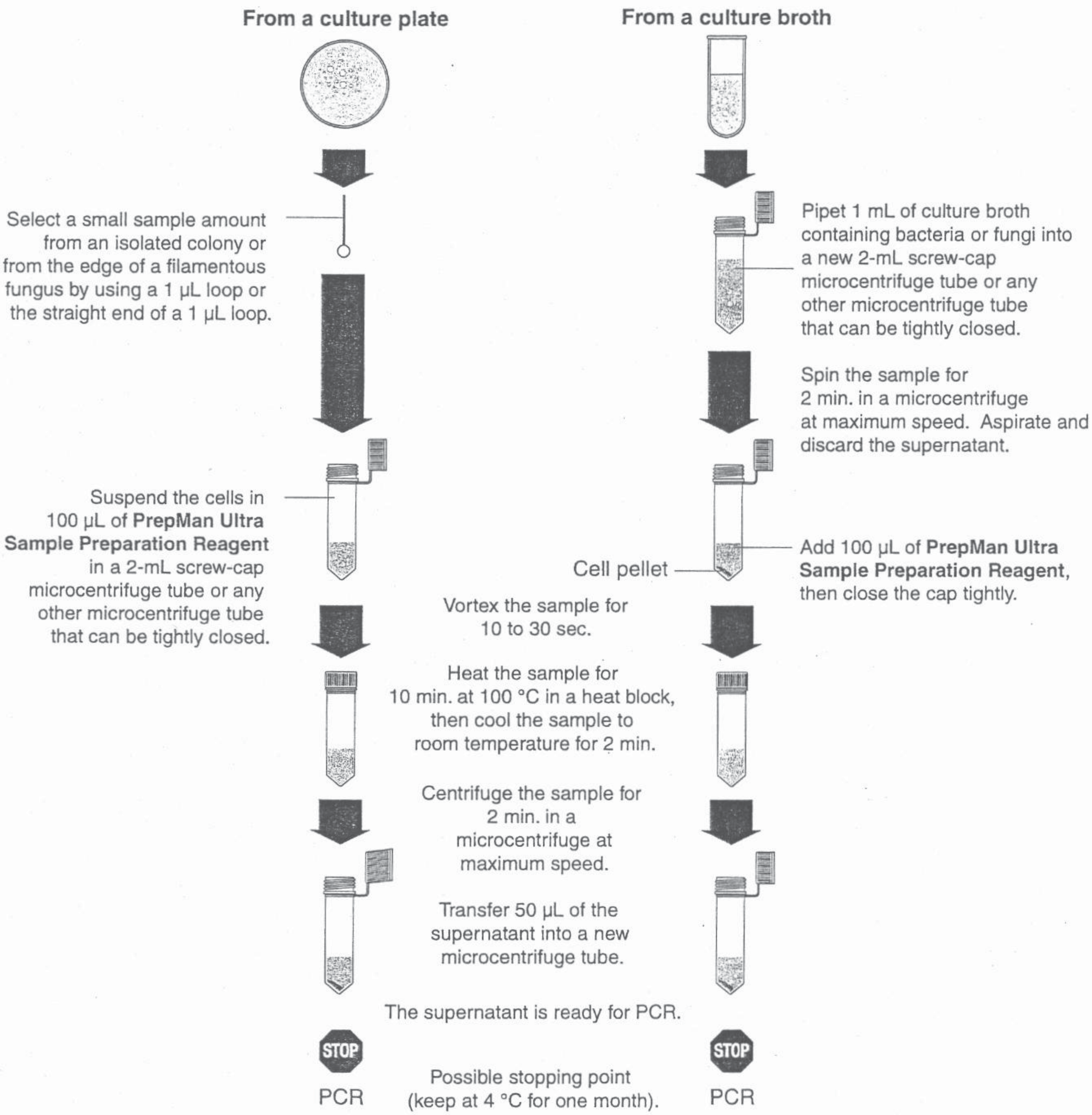

N.Join: 1.0%
POS
Escherichia coli (ATCC=35382)
Escherichia coli W3110 (Sigma=W3110)
Escherichia coli (ATCC=11303)

Quick Reference Card

For safety and biohazard guidelines, refer to the "Safety" section in the *PrepMan® Ultra Sample Preparation Reagent Protocol (PN 4367554)*. For every chemical, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

For a list of required and recommended equipment, consumables, and reagents refer to the *PrepMan® Ultra Sample Preparation Reagent Protocol (PN 4367554)*.

DNA Sample Preparation for Bacterial and Fungal Cultures



From a culture plate

Select a small sample amount from an isolated colony or from the edge of a filamentous fungus by using a 1 µL loop or the straight end of a 1 µL loop.

Suspend the cells in 100 µL of **PrepMan Ultra Sample Preparation Reagent** in a 2-mL screw-cap microcentrifuge tube or any other microcentrifuge tube that can be tightly closed.

From a culture broth

Pipet 1 mL of culture broth containing bacteria or fungi into a new 2-mL screw-cap microcentrifuge tube or any other microcentrifuge tube that can be tightly closed.

Spin the sample for 2 min. in a microcentrifuge at maximum speed. Aspirate and discard the supernatant.

Add 100 µL of **PrepMan Ultra Sample Preparation Reagent**, then close the cap tightly.

Vortex the sample for 10 to 30 sec.

Heat the sample for 10 min. at 100 °C in a heat block, then cool the sample to room temperature for 2 min.

Centrifuge the sample for 2 min. in a microcentrifuge at maximum speed.

Transfer 50 µL of the supernatant into a new microcentrifuge tube.

The supernatant is ready for PCR.

Possible stopping point (keep at 4 °C for one month).

FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit Protocol* (PN 4393007). For every chemical, read the SDS (Safety Data Sheet) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card provides brief procedures for using the MicroSEQ® Full Gene 16S rDNA Bacterial Identification Kit after you have isolated DNA using PrepMan® Ultra Sample Reagent. See the *FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit Protocol* and *Prepman® Ultra Sample Reagent Protocol* for details.

This quick reference card covers:

- MicroSEQ® System Overview 1
- Kit Contents 1
- Protocol 1

MicroSEQ® System Overview

The MicroSEQ® Microbial Identification System combines all of the instrumentation, reagents, sequence libraries, and software required for automated microbial identification using DNA sequencing.

The MicroSEQ® system is easy to use and suitable for the routine identification of all bacterial and fungal isolates, including organisms that are difficult to grow, non-viable, or unidentifiable using phenotypic methods. The MicroSEQ® system identifies bacterial and fungal isolates from a small sample of pure culture without preliminary testing or growth on selective media.

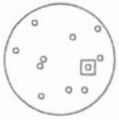
Kit Contents

The table below describes the contents of the two bacterial kits.

Kit	Component	Description
FAST MicroSEQ® 500 16S rDNA PCR Kit (PN 4370489)	FAST MicroSEQ® 500 16S PCR Master Mix	One tube containing 2X PCR Master Mix sufficient for 60 amplifications
	Positive Control	One tube of positive control DNA (*E. coli*) at 1 ng/µL, sufficient for 10 positive-control assays
	Negative Control	One tube of negative control (water)
MicroSEQ® 500 16S rDNA Sequencing Kit (PN 4346480)	MicroSEQ® 500 16S Forward Sequence Mix	Two tubes sufficient for a total of 55 reactions
	MicroSEQ® 500 16S Reverse Sequence Mix	Two tubes sufficient for a total of 55 reactions

Protocol

1 **Isolating Genomic DNA from Samples**



a. Isolate bacterial genomic DNA from bacterial colonies, using Applied Biosystems PrepMan® Ultra Sample Preparation Reagent. Follow the instructions in the *PrepMan® Ultra Sample Preparation Reagent Protocol.*

b. When the supernatant is not in use, Applied Biosystems recommends storing it at – 15 to – 25 °C. Before use, thaw, then vortex and centrifuge the stored supernatant. Alternatively, the supernatant can be covered and stored at 4 °C for up to 1 month.

2 Diluting Genomic DNA for PCR

a. Pipette 495 µL of nuclease-free water into a 1.5-mL microcentrifuge tube.

b. Add 5 µL of the PrepMan® Ultra supernatant.

> **Note:** If the PrepMan® Ultra supernatant is colored (typically a shade of black or green), PCR inhibition may occur. See "Troubleshooting" in the *FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit Protocol.*

c. When the supernatant is not in use, Applied Biosystems recommends storing it at – 15 to – 25 °C. Before use, thaw, then vortex and centrifuge the stored supernatant. Alternatively, the supernatant can be covered and stored at 4 °C for up to 1 month.

3 Preparing PCR Reactions

a. Vortex the diluted supernatant to mix the tube contents.

b. Using the volumes shown in the table, prepare samples and controls in MicroAmp® reaction tubes or 96-well plates.

Reaction Type	Volume for One Reaction
Negative controls	• 15 µL of FAST PCR Master Mix • 15 µL of negative control (provided with kit)
Samples	• 15 µL of FAST PCR Master Mix • 15 µL of 1:100 dilution of PrepMan® Ultra supernatant
Positive controls	• 15 µL FAST PCR Master Mix • 15 µL of positive-control DNA (provided with kit)

c. Use strip caps and the capping tool, or adhesive film and the sealing tool, to cap the tubes or plate. Vortex, spin briefly, then place the tubes or the plate in the thermal cycler.

4 Performing the Amplification Run

a. Set the appropriate ramp mode for your thermal cycler:

- Veriti® 96-Well Thermal Cycler — **Default**
- 9800 — **Fast (F96)**
- GeneAmp® PCR System 9700 — **Maximum (Max)**

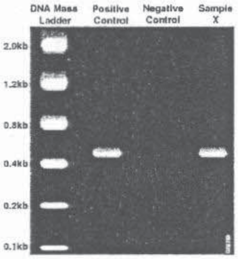
b. Set the thermal cycling conditions:

Initial Step	Each of 30 Cycles		Final Extension	Final Step
	Melt	Anneal		
HOLD	CYCLE		HOLD	HOLD
95 °C 10 sec	95 °C 0 sec	64 °C 15 sec	72 °C 1 min	4 °C ∞

c. Set the reaction volume to **30 µL**, then start the run.

d. Before removing the caps or adhesive film, briefly centrifuge the tubes or plate.

5 Analyzing PCR Products (Optional)



a. Load 5 µL of PCR product per lane on one of the following agarose gel separations:

- 2% agarose gel separation if you use another ready-to-use gel system (such as E-gel® from Invitrogen) or if you prepare your own gel
- 1.2% agarose gel separation if using a Lonza Bioscience FlashGel cassette

Note: Read and understand the SDSs provided by the manufacturer before you store, handle, or work with any chemicals or hazardous materials.

b. Use the Mass Standard Ladder to estimate the PCR product yield. In a positive control or sample, a single fragment ranging from 460 to 560 bp in size should be detected on a gel. Actual fragment size depends on the bacterial species. No product should be visible in a negative control reaction.

6 Purifying PCR Products for Cycle Sequencing

a. Remove unused dNTPs and primers from each PCR product using one of the following products:

- ExoSAP-IT® (USB)
- Montage® PCR Filter Unit (Millipore)

Note: Read and understand the SDSs provided by the manufacturer before you store, handle, or work with any chemicals or hazardous materials.

b. Cover and store the PCR products at – 15 to – 25 °C until you are ready to use them.

7 Preparing Cycle Sequencing Reactions

a. Before removing the tube or plate caps, briefly centrifuge the purified PCR products.

b. In reaction tubes or a 96-well plate, prepare separate forward- and reverse-sequencing reactions for each PCR product and control:

- **Forward-sequencing reaction** — Combine 7 µL of purified PCR product or control + 13 µL of the forward sequence mix
- **Reverse-sequencing reaction** — Combine 7 µL of purified PCR product or control + 13 µL of the reverse sequence mix

IMPORTANT! Select the appropriate tubes or 96-well plates for your thermal cycler. See your instrument user guide, or go to **www.microseq.com**, click **System** (under MicroSEQ in the right menu), then click **MicroSEQ® System List of Other Required Materials**.

c. Cover and store the unused portions of the purified PCR products at – 15 to – 25 °C until you are ready to use them.

d. Cap the tubes or the plate, then place the tubes or the plate in the thermal cycler.

8 Performing the Cycle Sequencing Run

a. Set the appropriate ramp mode for your thermal cycler:

- Veriti® 96-Well Thermal Cycler — **Default**
- 9800 — **Fast (F96)** thermal cycler
- GeneAmp® PCR System 9700 — **Maximum (Max)**

b. Set the thermal cycling conditions:

Initial Step	Each of 25 Cycles			Final Step
	Melt	Anneal	Extend	
HOLD	CYCLE			HOLD
96 °C 1 min	96 °C 10 sec	50 °C 5 sec	60 °C 1 min 15 sec	4 °C ∞

c. Set the reaction volume to **20 µL**, then start the run.

d. Before removing the tube or plate caps, briefly centrifuge the extension products.

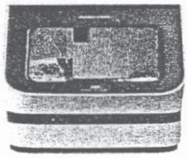
e. If necessary, cover and store the extension products at 4 °C overnight or at – 15 to – 25 °C for up to 1 week before purifying them.

9 Purifying Extension Products

For...	Purify using one of the following...
Tubes	• Performa® DTR Gel Filtration Cartridges (Edge Biosystems) • DyeEx® 2.0 Spin Kit (Qiagen) • CentriSep Column (Applied Biosystems)
96-well plates	• Performa® DTR Ultra 96-Well Plate Kit (Edge Biosystems) • DyeEx® 96 Kit (Qiagen)

Follow the guidelines and procedures supplied with the kits.

10 Performing Electrophoresis



IMPORTANT! If the electrophoresis run time will be longer than 24 hours (for example, if you are injecting more than 40 wells on a 4-capillary instrument or more than 160 wells on a 16-capillary instrument) evaporation might occur. Therefore, Applied Biosystems recommends the addition of formamide to the reactions. See Appendix B, "Preventing Evaporation During Electrophoresis" in the *FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit Protocol*.

a. Configure the instrument with the parameter settings in the following table.

Instrument	Procedure
3130/3130*xl*	Specify: • **Filter Set** – E • **Run Module** – StdSeq50_POP6_1 • **Base-caller** – KB.bcp • **DyeSet/Primer (Mobility File)** – KB_3130_POP6_BDT v1.mob
Applied Biosystems 3500/3500xL	Create a plate using the MSID plate template in the 3500 Series Data Collection software. This plate template contains an instrument protocol/run module (POP-6™ polymer) and a basecalling protocol optimized for MicroSEQ ID applications.

b. Prepare reactions:

1. Pipette at least 15 µL of each purified extension product or control into separate wells in a 96-well plate.
2. Pipette 15 µL of Hi-Di™ formamide into each blank well that will be injected together with samples.

Note: See the *FAST MicroSEQ® 500 16S rDNA Bacterial Identification Kit Protocol* for alternative loading options.

c. Cover the plate, centrifuge, then load the plate into your instrument. Start the run.

d. Cover and store the unused portion of the purified extension products at 4 °C overnight or at – 15 to – 25 °C for up to 1 week.

e. When the run is complete, review the data using the MicroSEQ® ID Analysis Software.

For Research Use Use Only. Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE FAST MICROSEQ® 500 16S RDNA BACTERIAL IDENTIFICATION KIT PRODUCT INSERT AND PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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Part Number 4393012 Rev. B 01/2010

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Exo -Sap

USB® ExoSAP-IT® PCR Product Cleanup
Product numbers 78200/01/02/05/50
Brief protocol

ExoSAP-IT reagent treats PCR products ranging in size from less than 100 bp to over 20 kb with absolutely no sample loss by removing unused primers and nucleotides.

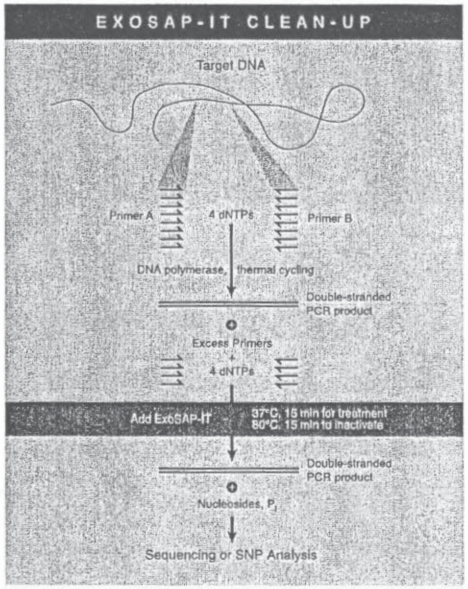
Add ExoSAP-IT reagent directly to the reaction products following PCR. ExoSAP-IT PCR Product Cleanup is active in commonly used PCR buffers, so no buffer exchange is required. After treatment, ExoSAP-IT reagent is inactivated by heating to 80°C for 15 minutes. The treated PCR products are now ready for subsequent analysis in applications that require DNA to be free of excess primers and nucleotides.

PCR cleanup protocol:

1. Remove ExoSAP-IT reagent from -20°C freezer and keep on ice throughout this procedure.
2. Mix 5 µl of a post-PCR reaction product with 2 µl of ExoSAP-IT reagent for a combined 7 µl reaction volume. Note: When treating PCR product volumes greater than 5 µl, simply increase the amount of ExoSAP-IT reagent proportionally.
3. Incubate at 37°C for 15 minutes to degrade remaining primers and nucleotides.
4. Incubate at 80°C for 15 minutes to inactivate ExoSAP-IT reagent.
5. The PCR product is now ready for use in DNA sequencing, SNP analyses, or other primer-extension applications. Treated PCR products may be stored at -20°C until required.

Note: Store ExoSAP-IT reagent in a non-frost-free freezer.

Affymetrix® | USB® Products


EXOSAP-IT CLEAN-UP
Target DNA
Primer A
4 dNTPs
Primer B
DNA polymerase, thermal cycling
Double-stranded PCR product
Excess Primers
4 dNTPs
Add ExoSAP-IT
37°C, 15 min for treatment
80°C, 15 min to inactivate
Double-stranded PCR product
Nucleosides, Pᵢ
Sequencing or SNP Analysis

USB and ExoSAP-IT are registered trademarks of Affymetrix, Inc.
ExoSAP-IT is covered by US Patent Nos. 6,379,940 and 6,387,634.
Exonuclease I/Shrimp Alkaline Phosphatase—This product is licensed under US Patent Nos. 5,741,676 and 5,756,285, and corresponding patents issued in other countries. Purchase of this product includes a license to use this product in a restricted way within the scope of rights granted to USB by GE Healthcare. No other license is granted to the purchaser either directly or by implication, estoppel or otherwise.
The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F. Hoffman-La Roche Ltd.

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usb.affymetrix.com
P-78200B
rev 10/10

EdgeBio

Product Summary

PERFORMA® DTR Gel Filtration Cartridges

Product	Catalog #	Purifications
Performa DTR Gel Filtration Cartridges (36 cartridges)	98780	36
Performa DTR Gel Filtration Cartridges (108 cartridges)	42453	108

Description

Performa DTR (Dye Terminator Removal) Gel Filtration Cartridges are 0.8-ml spin columns packed with a gel matrix optimized to effectively remove dye terminators, dNTPs, and other low molecular weight materials from sequencing reactions. These columns also remove DNA primers and fragments up to 15 bases, buffers, and nucleotides labeled with biotin, isotopes and other assorted markers. The column is pre-packed with the matrix fully hydrated in water.

Components	98780	42453
Performa Gel Filtration Cartridges	36 carts. (PN 4050167)	108 carts. (3 x PN 4050167)
1.5-ml Microcentrifuge Tubes	36 tubes (PN 4050090)	108 tubes (PN 4050087)

Equipment and Materials Required

1. Variable speed centrifuge (benchtop or floor model) capable of 850 *x g*.
2. Carriers for microcentrifuge tubes.

Storage Condition

Store at +4°C. Do not freeze.

Quality Control

Tested for sequence quality, including signal strength, removal of fluorescent contaminants, and sequencing accuracy on a gel sequencer.

Recommended Protocol for use with BigDye™ v3.1

1. **Centrifuge the Performa Gel Filtration Cartridge for 3 minutes at 850 *x g*.**
   - The time and speed of centrifugation are important.
   - The drier the packing (longer centrifugation times and/or higher *g* forces), the longer it takes to recover product and the lower the overall recovery.
   - Conversely, shorter spin times and lower speeds result in elution volumes higher than the input sample volume.
   - See "Notes" for determination of RPM from RCF or visit our website at www.edgebio.com and click on Technical Support.
2. **Transfer the cartridge to the provided 1.5-ml microcentrifuge tube and add the sample to the packed column. Be sure the fluid runs into the gel.**
   - If using a microcentrifuge or other centrifuge which uses a fixed angle rotor, place the sample in the center of the slanted gel bed surface to obtain optimal performance.
3. **Close the cap and centrifuge for 3 minutes at 850 *x g*. Retain eluate.**
   - Up to 4 µl may be lost during sample processing.
   - If the volume loss is greater than 4 µl, this is an indication of an overly dry gel. To optimize recovery of sample, repeat centrifugation.

Recommended Protocol for all other Dye Terminators

1. **Centrifuge the Performa Gel Filtration Cartridge for 2 minutes at 750 x *g*.**
2. **Transfer the cartridge to the provided 1.5-ml microcentrifuge tube and add the sample to the packed column. Be sure the fluid runs into the gel.**
3. **Close the cap and centrifuge for 2 minutes at 750 x *g*. Retain eluate.**

Warning: This product is intended for **research use only**. It is not to be used for diagnostic purposes in humans or animals.

v.17

FCC Section Results Sheet

Sample No. 789348

TITLE: Microbiology (USP 71-Sterility Tests)

PURPOSE: To isolate and identify any aerobic organisms in the contents of Items 4, 5A, 6, 7A, 8, and 9A.

RESULTS (SUMMARY)

A portion from Items 4, 5A, 6, 7A, 8, and 9A were diluted in Trypticase Soy Broth and monitored for growth.

No aerobic bacteria were isolated from the contents of Item 4, 6, 7A, 8, and 9A.

All positive and negative controls performed as expected.

On February 27, 2013, one mold isolate from Item 5A was sent to CDC for identification.

Analyst:	Checked by:	Report Author:
Electronically signed by: Katie L. Korchinski (FCCLABS\kkorchinski) Date: Thursday, March 21, 2013 2:10:40 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski) Date: Thursday, March 21, 2013 2:11:44 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above	Electronically signed by: David L. Craft (FCCLABS\dcraft) Date: Thursday, May 02, 2013 1:39:40 PM (GMT -04:00) Location: FCC Reason: 01. Reason given above

FCC Continuation Sheet

Sample No. 789348

Method: USP 71 Sterility Tests – used as guidance following the instructions for Direct Inoculation of the Culture medium. Verison date: December 1, 2011
“FDA Sterility Testing Protocol for Ameridose Samples for Fungal and Other Growth. Version 2. 10/25/2012”.

Equipment and reagents:
Tryptic Soy Broth 90 ml (TSB); Ref R112840; Lot 235143; Exp date 2013/02/12
Tryptic Soy Broth 10 ml (TSB); Ref R07226; Lot 261484; Exp date 2013/10/17
Tryptic Soy Agar slant; R064862; Lot 248724; Exp date 2013/09/18
Sabouraud Dextrose Agar slant; R08722; Lot 252333; Exp date 2013/09/26

Quanti-cult; *Aspergillus brasiliensis*; Ref #R4731100; Lot #243449; Exp 2014/03/31; ATCC 16404
Quanit-cult; *Bacillus subtilis*; Ref #R4731221; Lot #195660; Exp 2013/11/30; ATCC 6633
Quanit-cult; *Candida albicans*; Ref #R4731503; Lot #097538; Exp 2013/03/31; ATCC 10231

Items 4, 5A, 6, 7A, 8, 9A

Sample preparation: 1.8.13 and 1.16.13

1. 10 ml from each Item was added to a 90 ml jar of TSB.
2. The jars were incubated at 25°C and monitored for 14 days.
3. An uninoculated jar of TSB was also incubated at 25°C as a negative control.
4. Growth was observed in NC on 1.14.12.
5. All jars were discarded.

Sample preparation: 1.14.13 – 1.16.13

1. New TSB jars were incubated at 35°C and monitored for two days.
2. The New TSB jars showed no growth after two days and on 1.16.12, 10 ml from each Item was added to a 90 ml jar of TSB.
3. The jars were incubated at 25°C and monitored for 14 days.
4. An uninoculated jar of TSB was also incubated at 25°C as a negative control.
5. Item 5A showed signs on growth on the 7th day and was stored at 4°C.
6. Items 4, 6, 7A, 8, 9A and the negative control showed no signs of growth.

Growth Promotion Test: 1.30.13 – Items 4 and 6

1. Three - 10 ml portions of TSB and sample were inoculated with *Aspergillus brasiliensis, Bacillus subtilis,* and *Candida albicans*
2. Three – 10 ml TSB tubes without sample were inoculated with *Aspergillus brasiliensis, Bacillus subtilis,* and *Candida albicans.*
3. One uninoculated TSB tube was used as a negative control.
4. Tubes were incubated at 25°C and monitored for 3-5 days.
5. Turbidity was shown in the growth promotion tubes and the negative controls worked as expected.

Item 5A Growth – 2.4.13
Three Sabouraud Dextrose Agar slants and two Tryptic Soy Agar slants were inoculated from the growth seen in Item 5A. The slants were incubated at 25°C and monitored for 3-5 days.

2.11.13 – No growth was shown on any of the slants inoculated on 2.4.13. The slants were re inoculated from the growth seen in Item 5A. The slants were incubated at 25°C and monitored for 3-5 days.

2.27.13 - One mold isolate on a SAB slant was sent to CDC for identification based upon the “FDA Sterility Testing Protocol for Ameridose Samples for Fungal and Other Growth. Version 2. 10/25/2012”.

FCC Analyst Worksheet

2. Sample No. 789348

1. CASE FILE NAME:
Ameridose Methylprednisolone

3. SEALS: ☒ Intact ☐ None ☐ Broken

3a. SEAL INSCRIPTION: "BMC 12-11-12"

4. RECEIVED FROM: Steve Gallagher

5. DATE RECEIVED: 1/7/2013

6. DESCRIPTION OF SAMPLE

General: ☒ Verified as on CR/CIE/Other Doc.
☐ Additional Description/Modification

Codes: ☒ Verified as on CR/CIE/Other Doc.
☐ None
☐ Provided, Section:

Condition of Sample: ☐ Frozen ☐ Cold ☒ Room Temp.

Photodocumentation: ☐ None Submitted ☒ Provided, Section: 1

7. BROKE SEAL ☒

Additional Description:

8. TABLE OF CONTENTS SECTION TITLE	SECTION	NO. PAGES
Microbiological Analysis	523	2
Microbiology: USP 61: Examination of Nonsterile Products	524	1
Microbiology	524a	19
Microbiology (USP 71-Sterility Tests)	715	2

9. Reserve Sample:
The remainder of items 1A and 1B identified, "789348 Item 1A…1B 1/15/2013 DLC" and in the original packaging further identified "789348 1/15/13 DLC" were placed in a heat-sealable bag identified "789348 5/2/13 DL Craft" and heat sealed. The heat seal was identified "DLC" and officially sealed "789348 5/12/13 David L. Craft". The remainder of item 3 identified, "789348 Item 3A 1/07/2013 DLC" and in the original packaging further identified "789348 1/7/13 DLC" was placed in a heat-sealable bag identified "789348 5/2/13 DL Craft" and heat sealed. The heat seal was identified "DLC" and officially sealed "789348 5/12/13 David L. Craft". The remainder of items 4, 5A. 6, 7A, 8 and 9A identified, "789348 Item 4…..9A 1/08/2013 DLC" and in the original packaging further identified "789348 1/8/13 DLC" were placed in a heat-sealable bag identified "789348 5/2/13 DL Craft" and heat sealed. The heat seal was identified "DLC" and officially sealed "789348 5/12/13 David L. Craft".The remainder of items 5B and 5C identified, "789348 Item 5B …..5C 4/09/2013 DLC" and in the original packaging further identified "789348 Item 5B…5C 4/9/2013 DLC" were placed in a

Analyst:

Electronically signed by: David L. Craft (FCCLABS\dcraft)
Date: Thursday, May 02, 2013 1:35:39 PM (GMT -04:00)
Location: FCC
Reason: 01. Reason given above

Checked by:

Electronically signed by: Jennifer Brzezinski (FCCLABS\jbrzezinski)
Date: Thursday, May 02, 2013 1:36:53 PM (GMT -04:00)
Location: FCC
Reason: 01. Reason given above

heat-sealable bag identified "789348 5/2/13 DL Craft" and heat sealed. The heat seal was identified "DLC" and officially sealed "789348 5/12/13 David L. Craft".

Analyst:	Checked by:

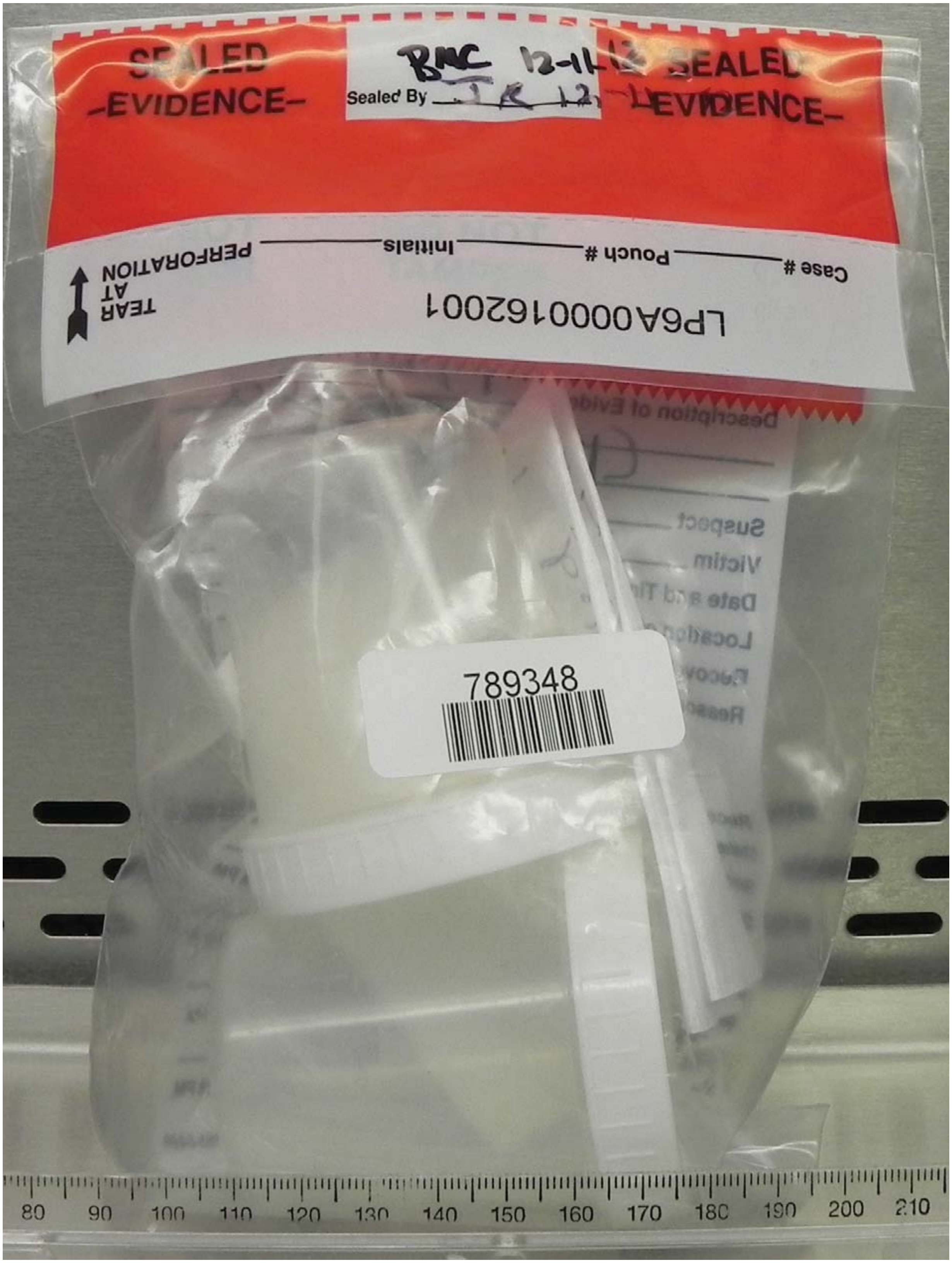


Fig. 1. Item 1 as received
Scale in mm

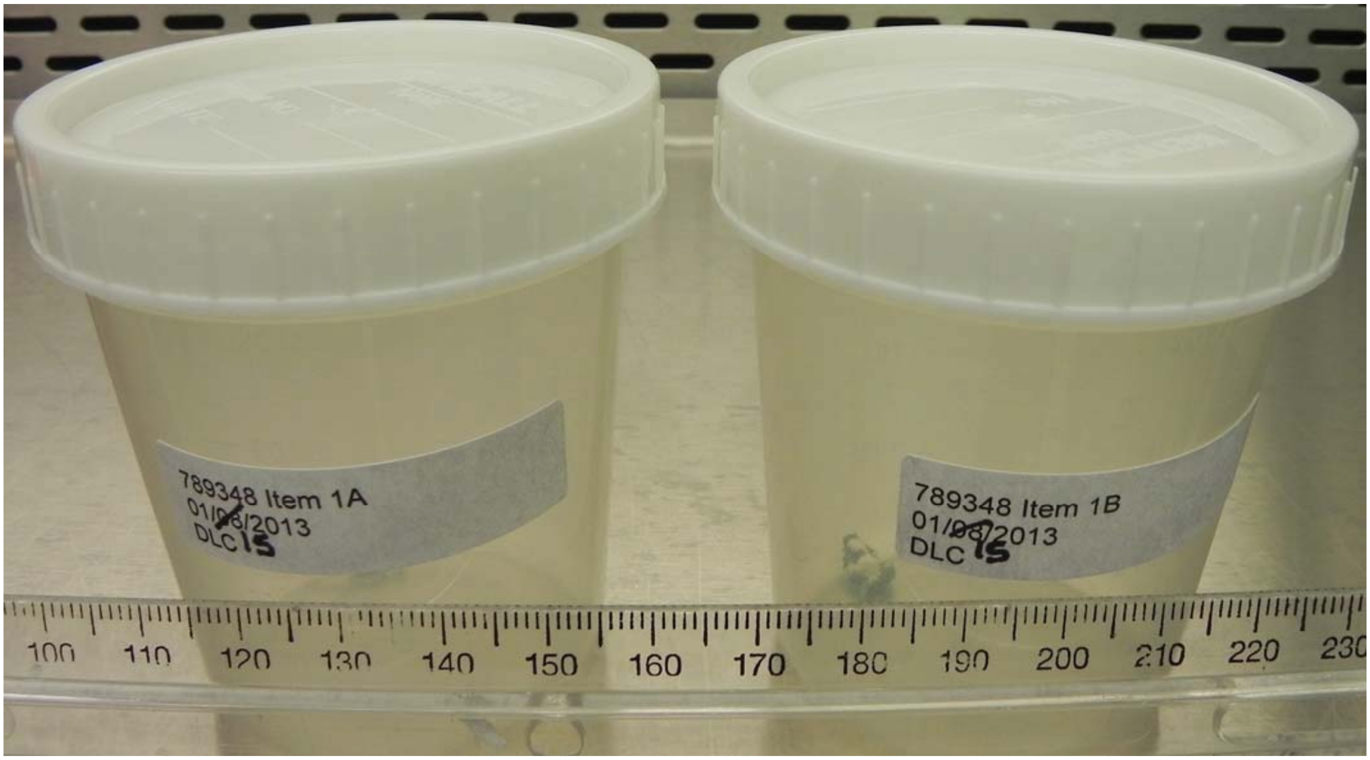
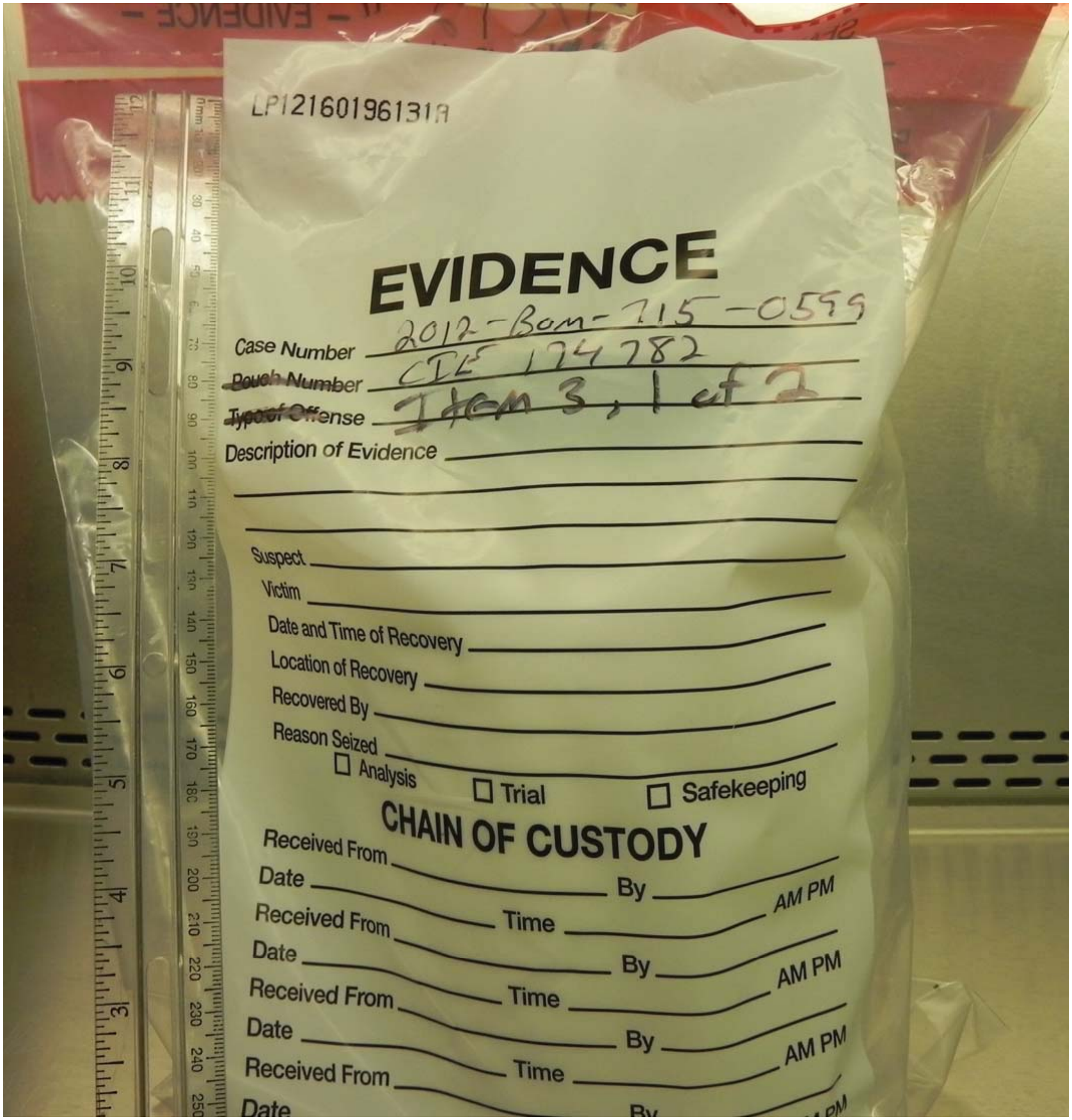


Fig. 2. Items 1A and 1B
Scale in mm



LP121601961318

EVIDENCE

Case Number 2012-Bom-715-0599

~~Pouch~~ Number CIE 174782

~~Type of~~ Offense Item 3, 1 of 2

Description of Evidence

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From

Date Time By AM PM

Received From

Date Time By AM PM

Received From

Date Time By AM PM

Received From

Date By

EVIDENCE
mm 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
11 10 9 8 7 6 5 4 3

Fig. 3. Item 3 as received
Scale in mm

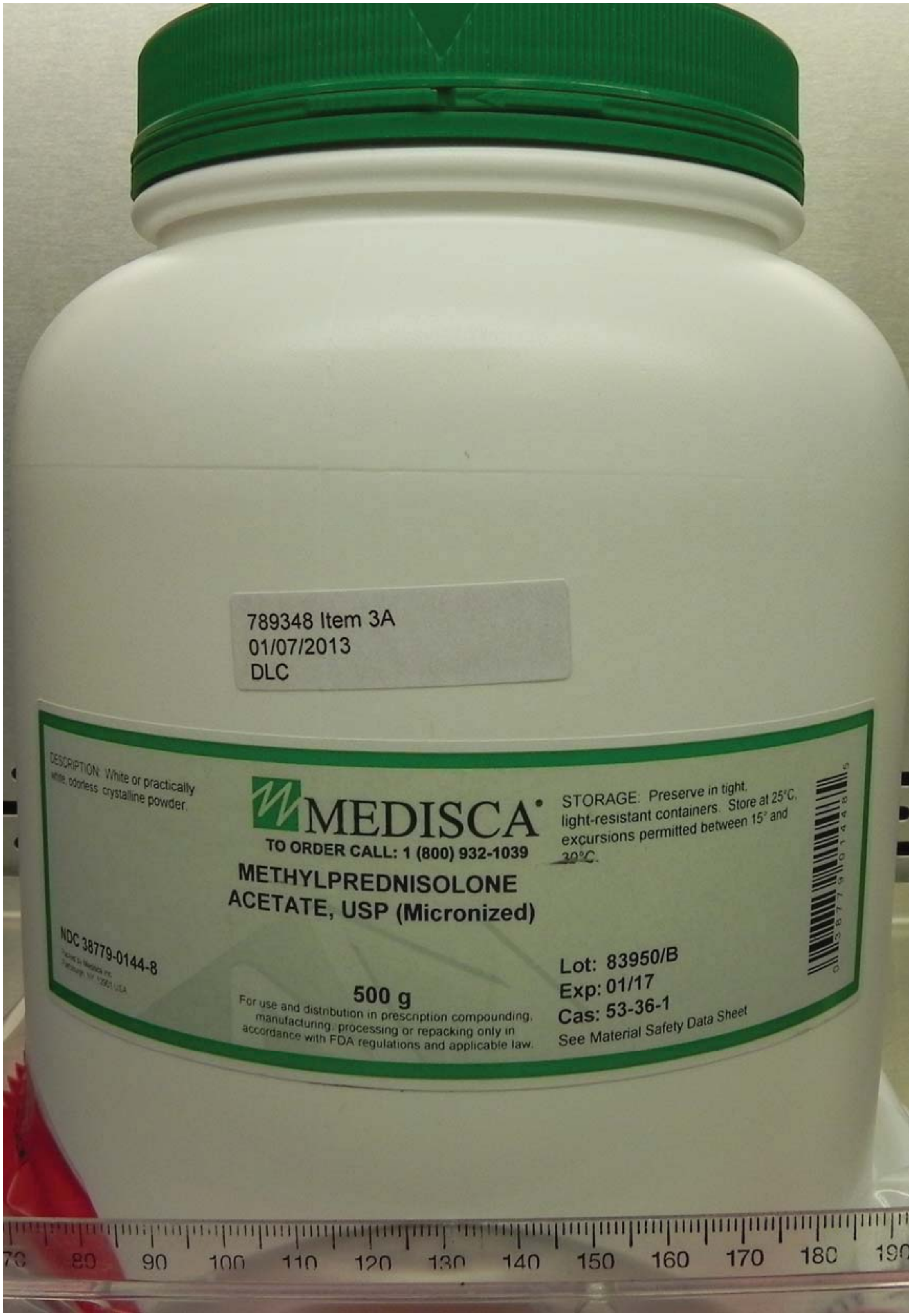
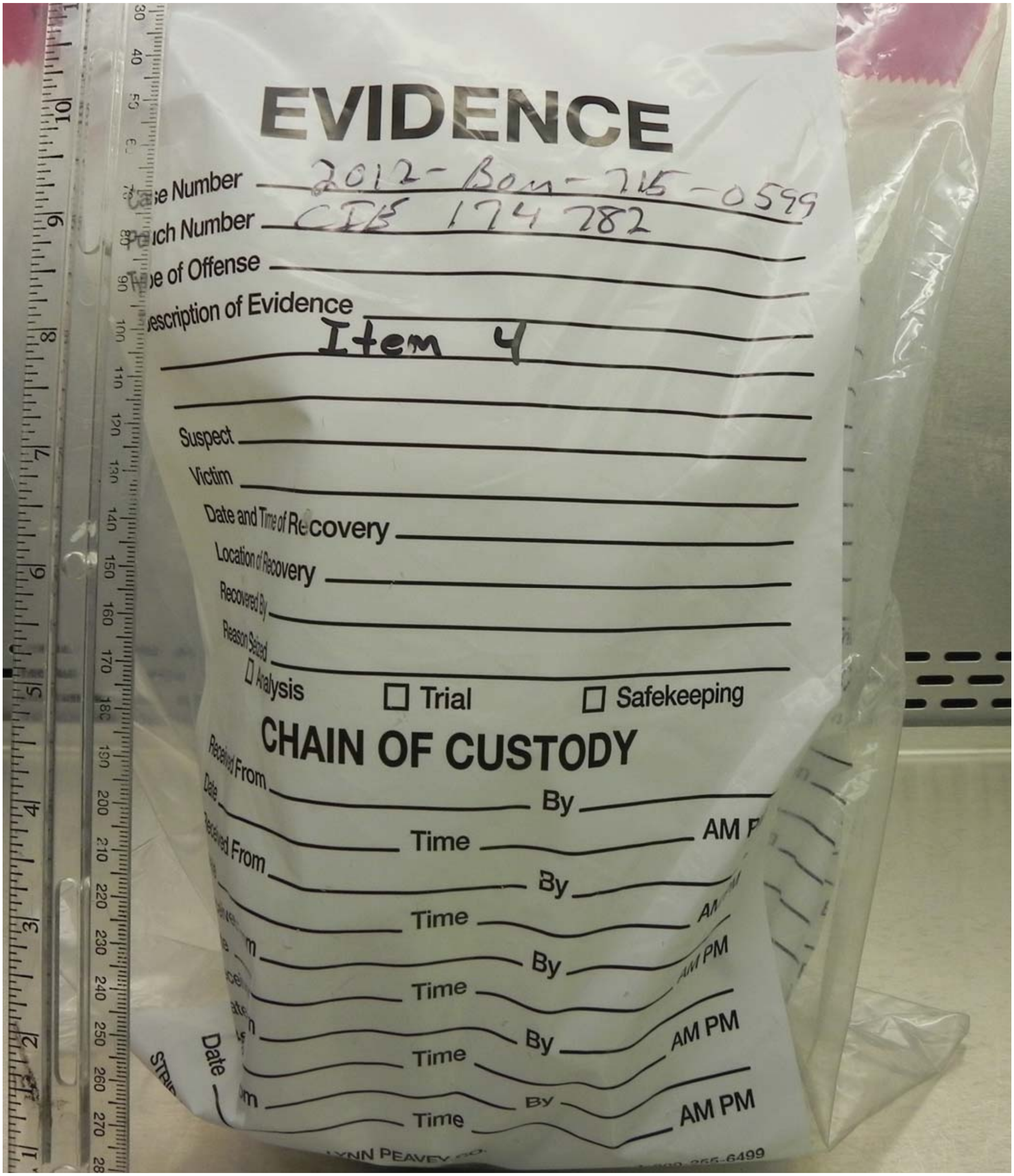

789348 Item 3A
01/07/2013
DLC
DESCRIPTION: White or practically white, odorless crystalline powder.
MEDISCA
TO ORDER CALL: 1 (800) 932-1039
METHYLPREDNISOLONE ACETATE, USP (Micronized)
STORAGE: Preserve in tight, light-resistant containers. Store at 25°C, excursions permitted between 15° and 30°C.
NDC 38779-0144-8
500 g
For use and distribution in prescription compounding, manufacturing, processing or repacking only in accordance with FDA regulations and applicable law.
Lot: 83950/B
Exp: 01/17
Cas: 53-36-1
See Material Safety Data Sheet
70
80
90
100
110
120
130
140
150
160
170
180
190

Fig. 4. Representative Item 3 (3A)
Scale in mm



EVIDENCE

ase Number 2012-Bos-715-0599

uch Number CDB 174 782

e of Offense

escription of Evidence

Item 4

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From By

Date Time AM P

eceived From By

Time AM

By AM PM

Time

By AM PM

Date Time

By AM PM

Time

Fig. 5. Item 4 as received
Scale in mm

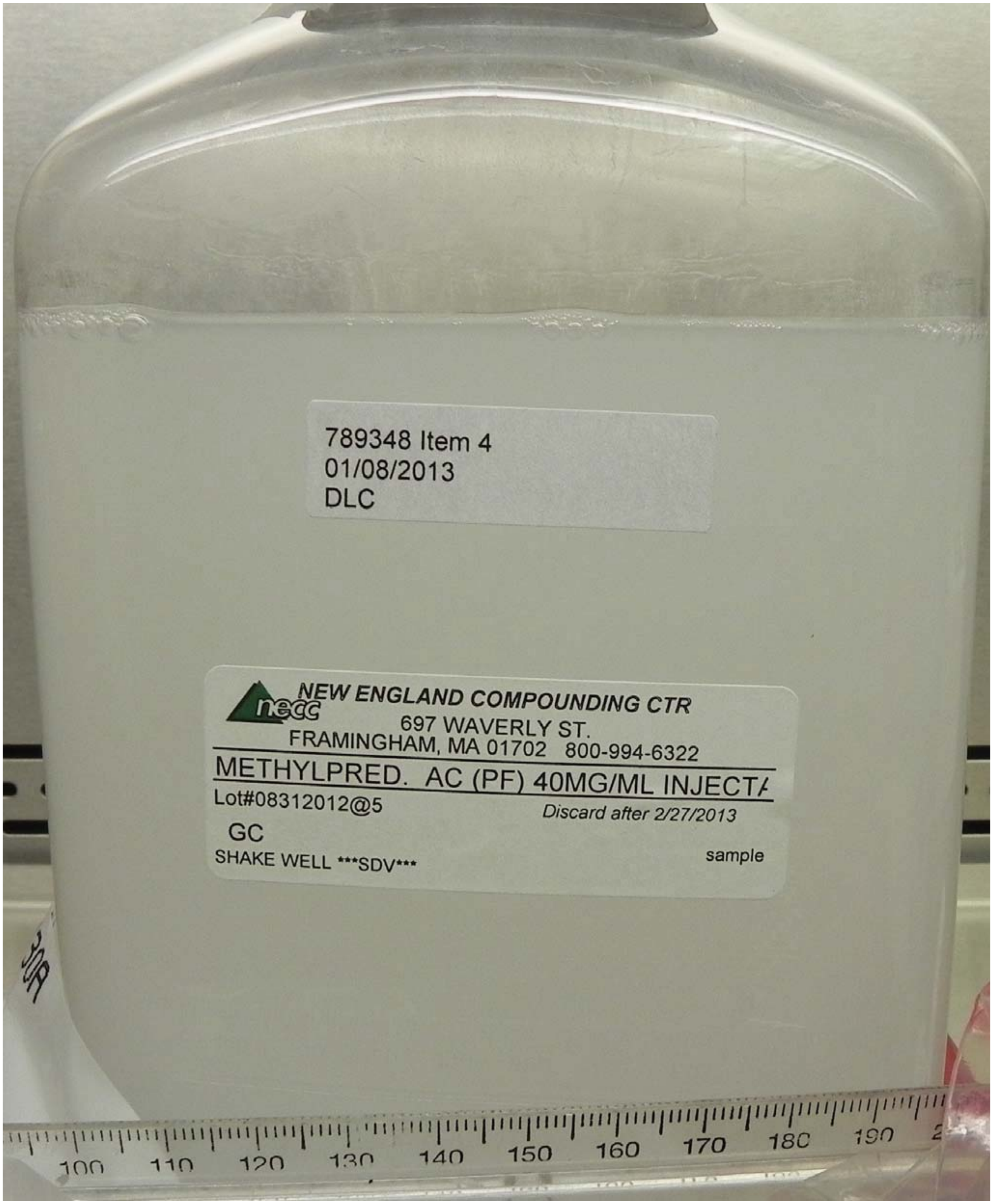
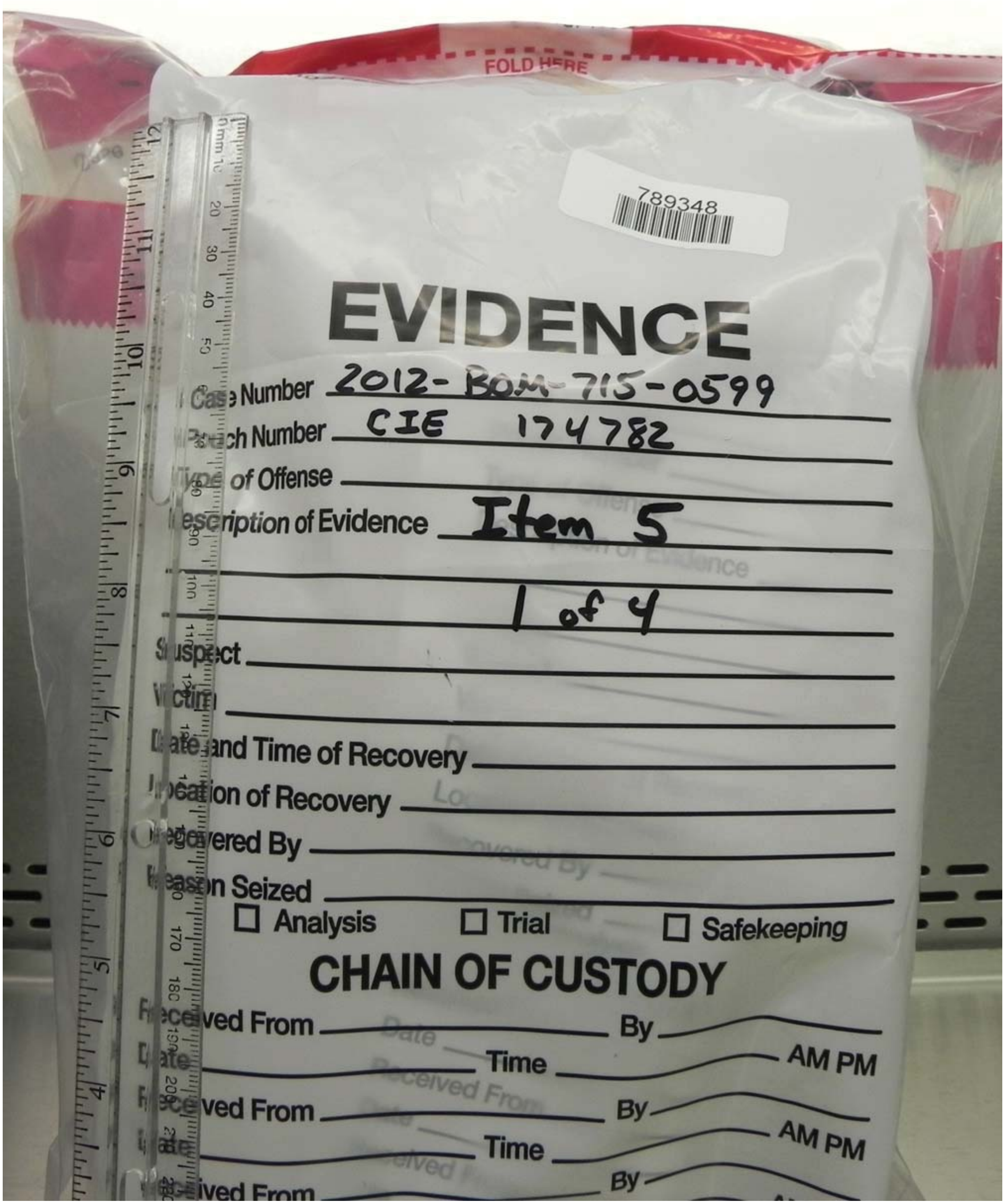


Fig. 6. Item 4
Scale in mm



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789348

EVIDENCE

Case Number 2012-BOM-715-0599

Pouch Number CIE 174782

Type of Offense

Description of Evidence Item 5

1 of 4

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From ______ By ______

Date ______ Time ______ AM PM

Received From ______ By ______

Date ______ Time ______ AM PM

Received From ______ By ______

Fig. 7. Item 5 as received
Scale in mm

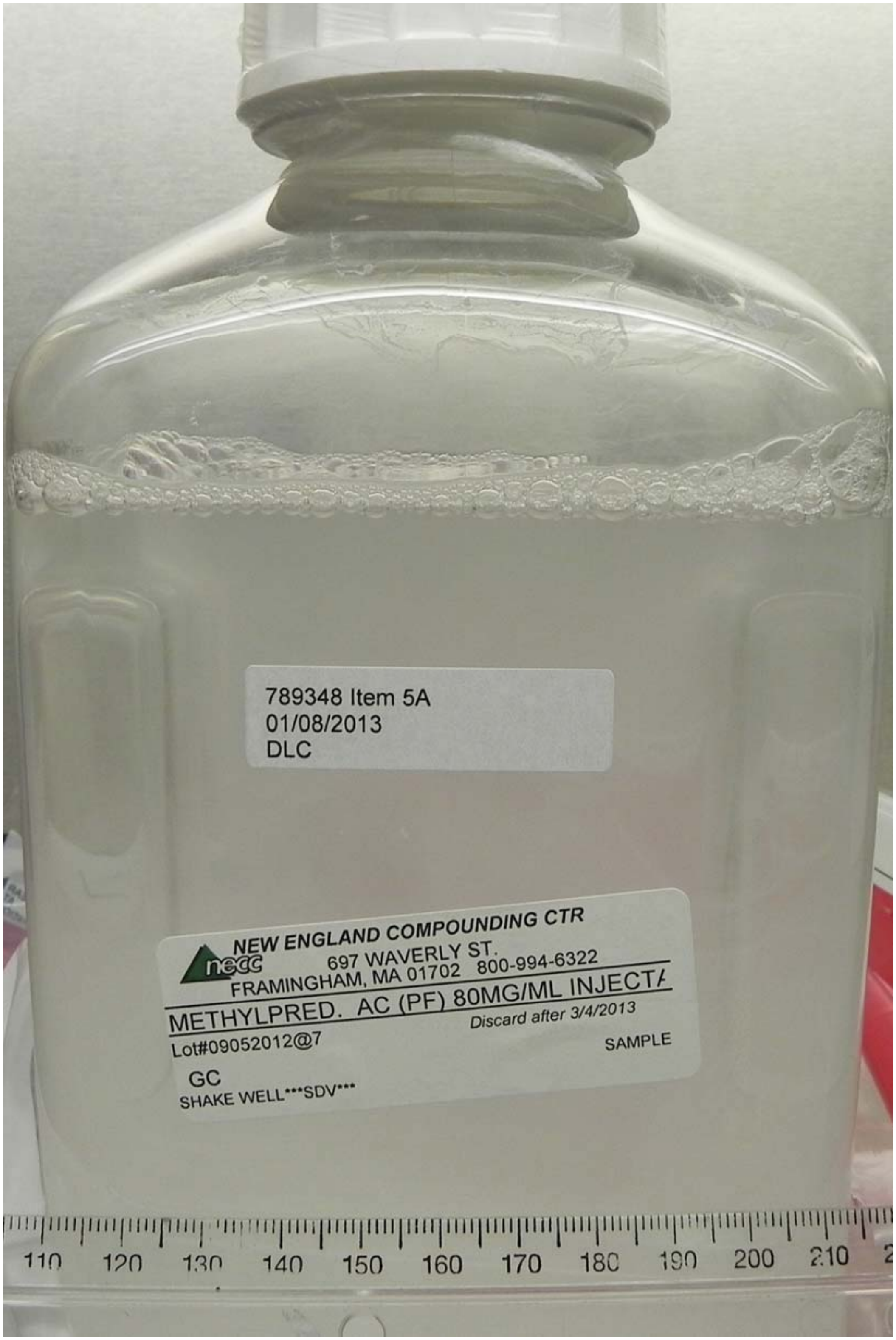
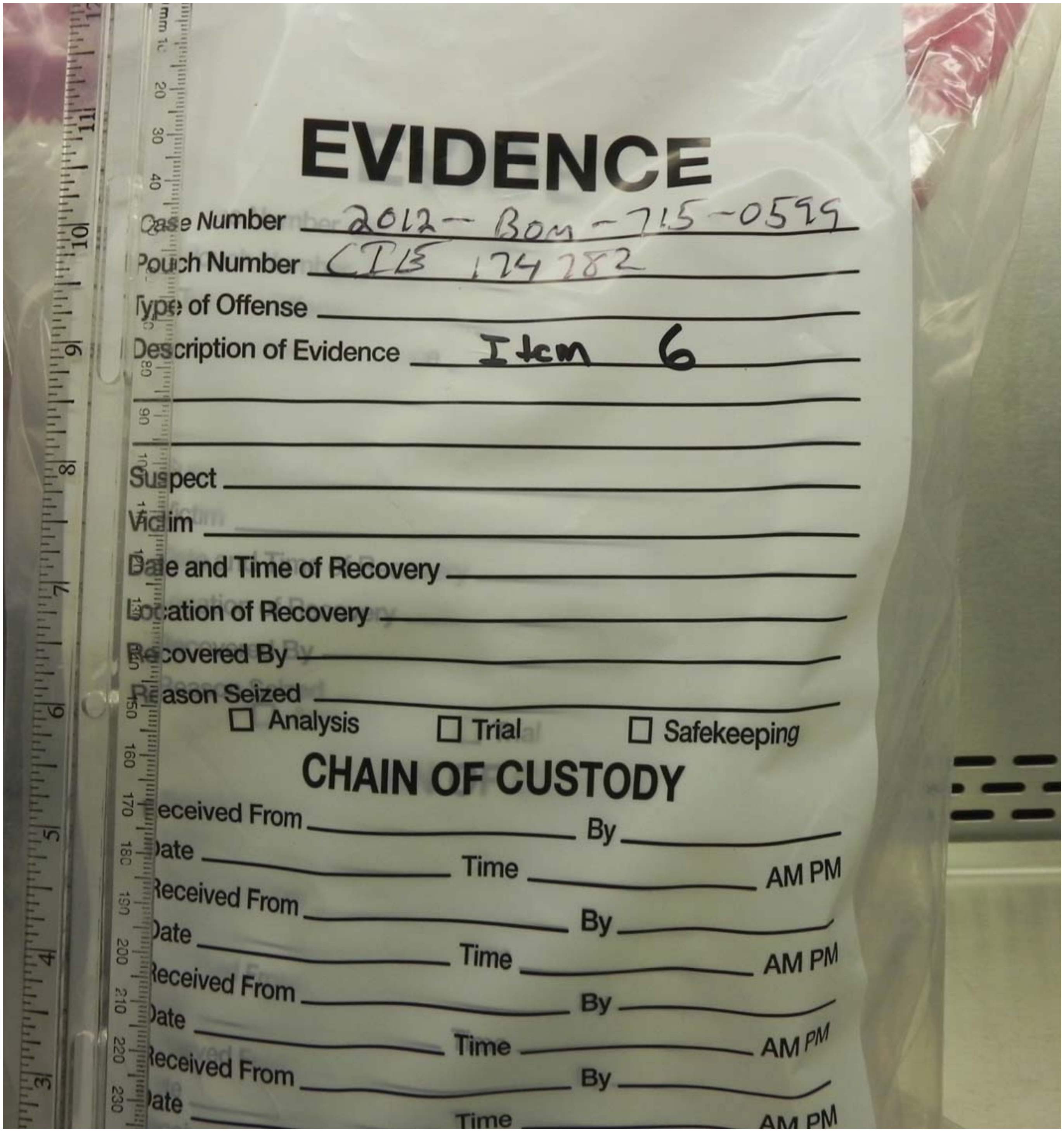


Fig. 8. Representative Item 5 (5A)
Scale in mm



EVIDENCE

Case Number 2012-BOM-715-0599

Pouch Number CIB 174282

Type of Offense

Description of Evidence Item 6

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From By

Date Time AM PM

Received From By

Date Time AM PM

Received From By

Date Time AM PM

Received From By

Date Time AM PM

Fig. 9. Item 6 as received
Scale in mm

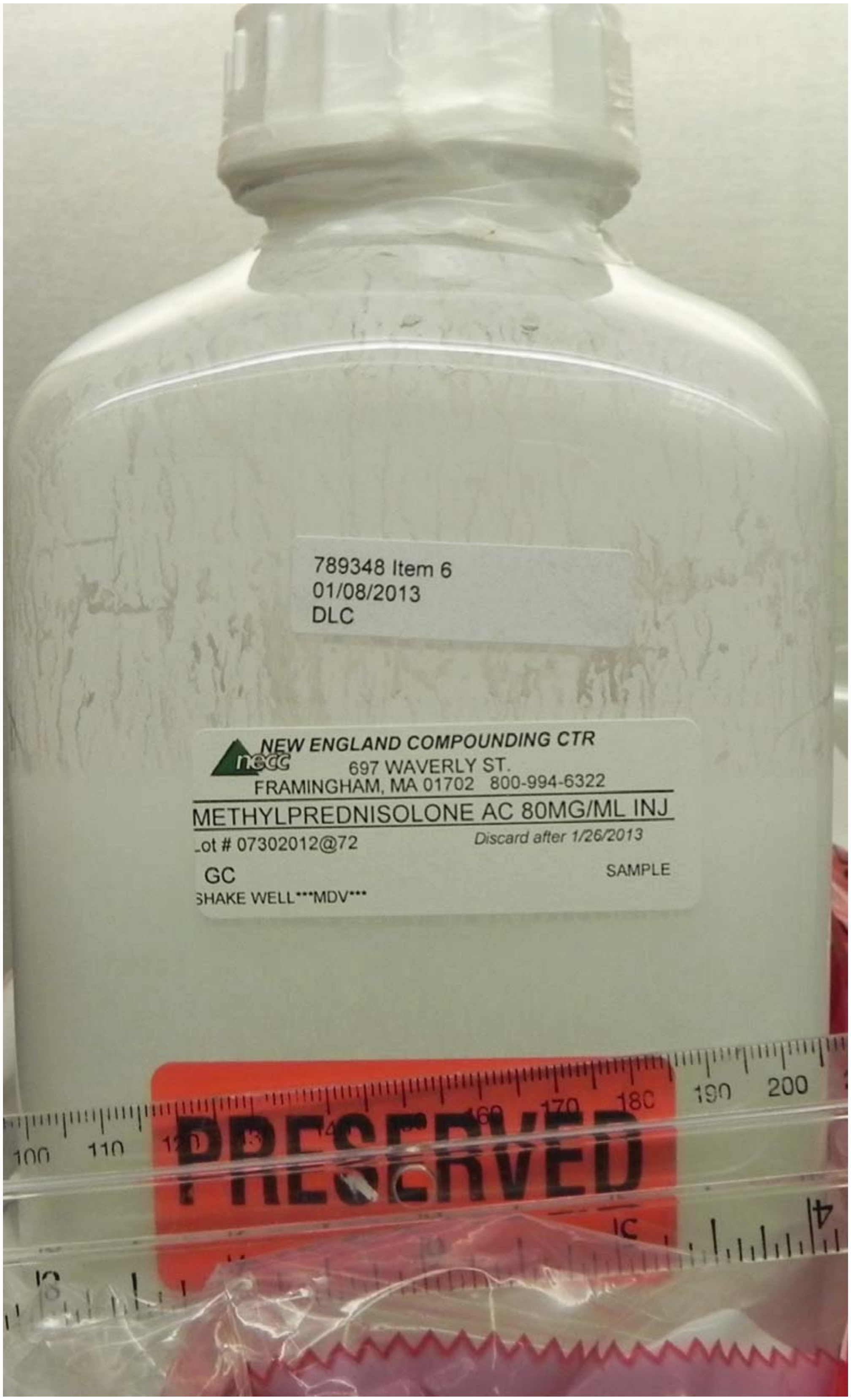
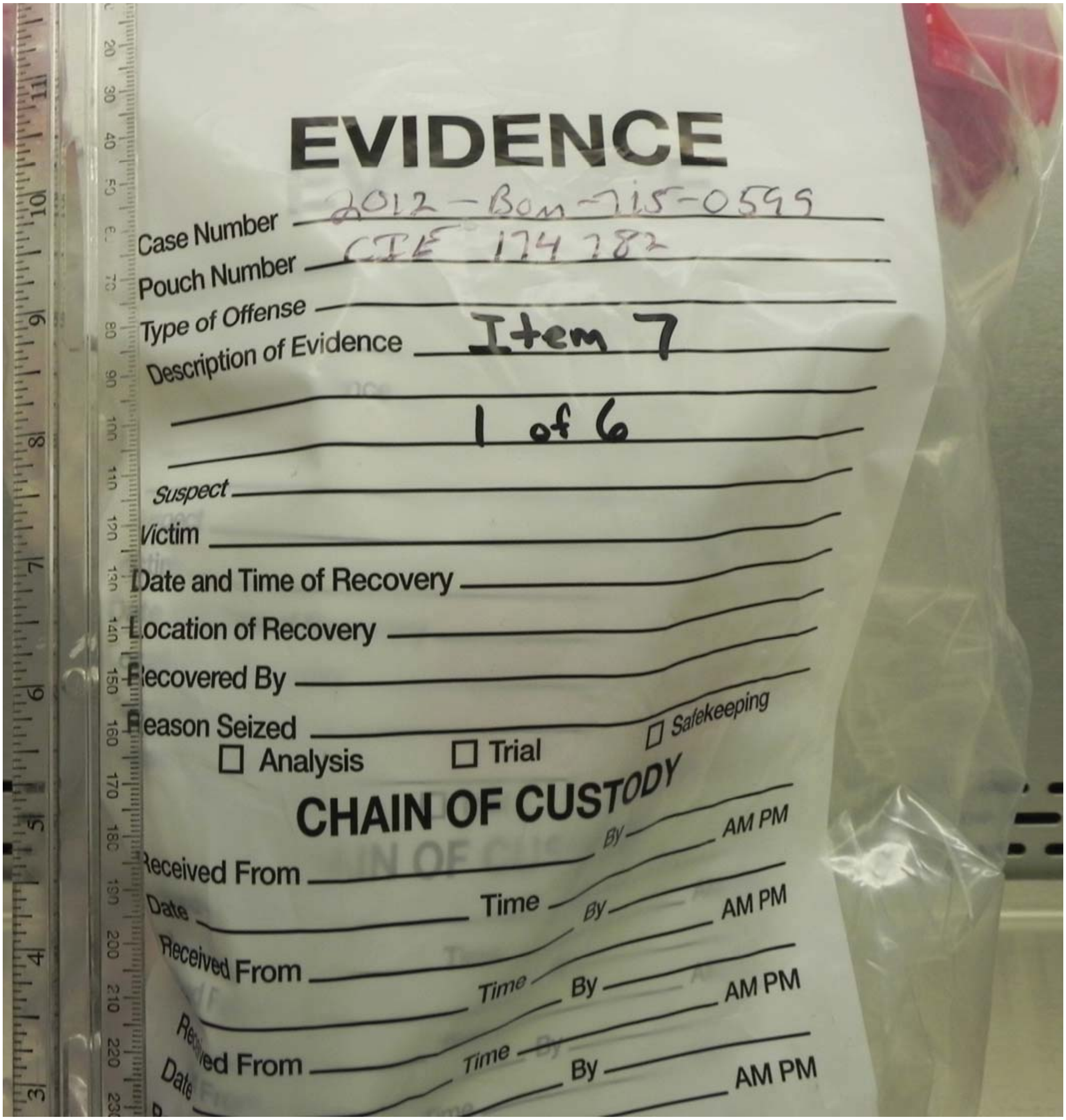


Fig. 10. Item 6
Scale in mm



EVIDENCE

Case Number 2012-Bom-715-0599

Pouch Number CIE 174782

Type of Offense

Description of Evidence Item 7

1 of 6

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From By AM PM

Date Time By AM PM

Received From

Time By AM PM

Received From

Date Time By AM PM

Fig. 11. Item 7 as received
Scale in mm

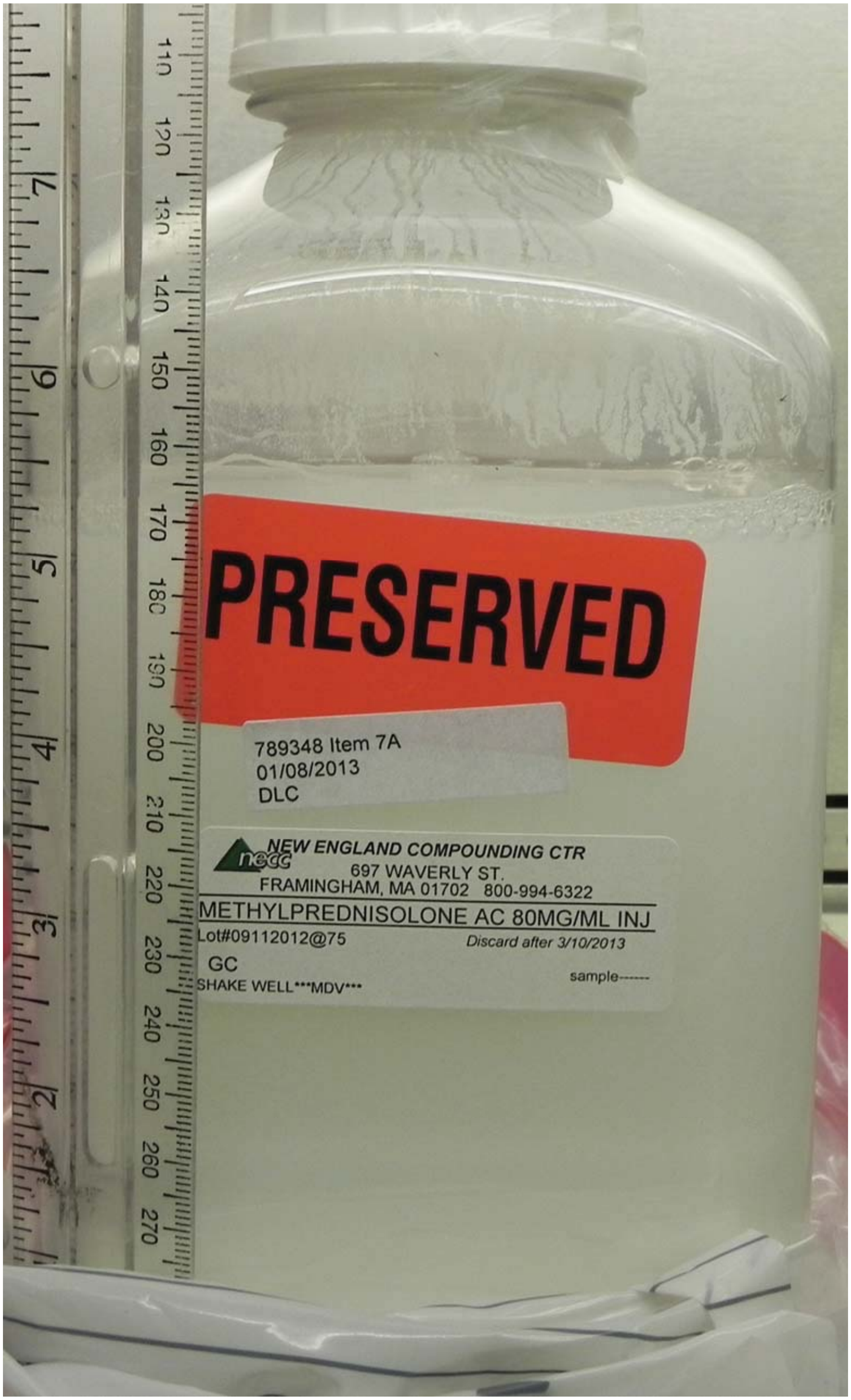
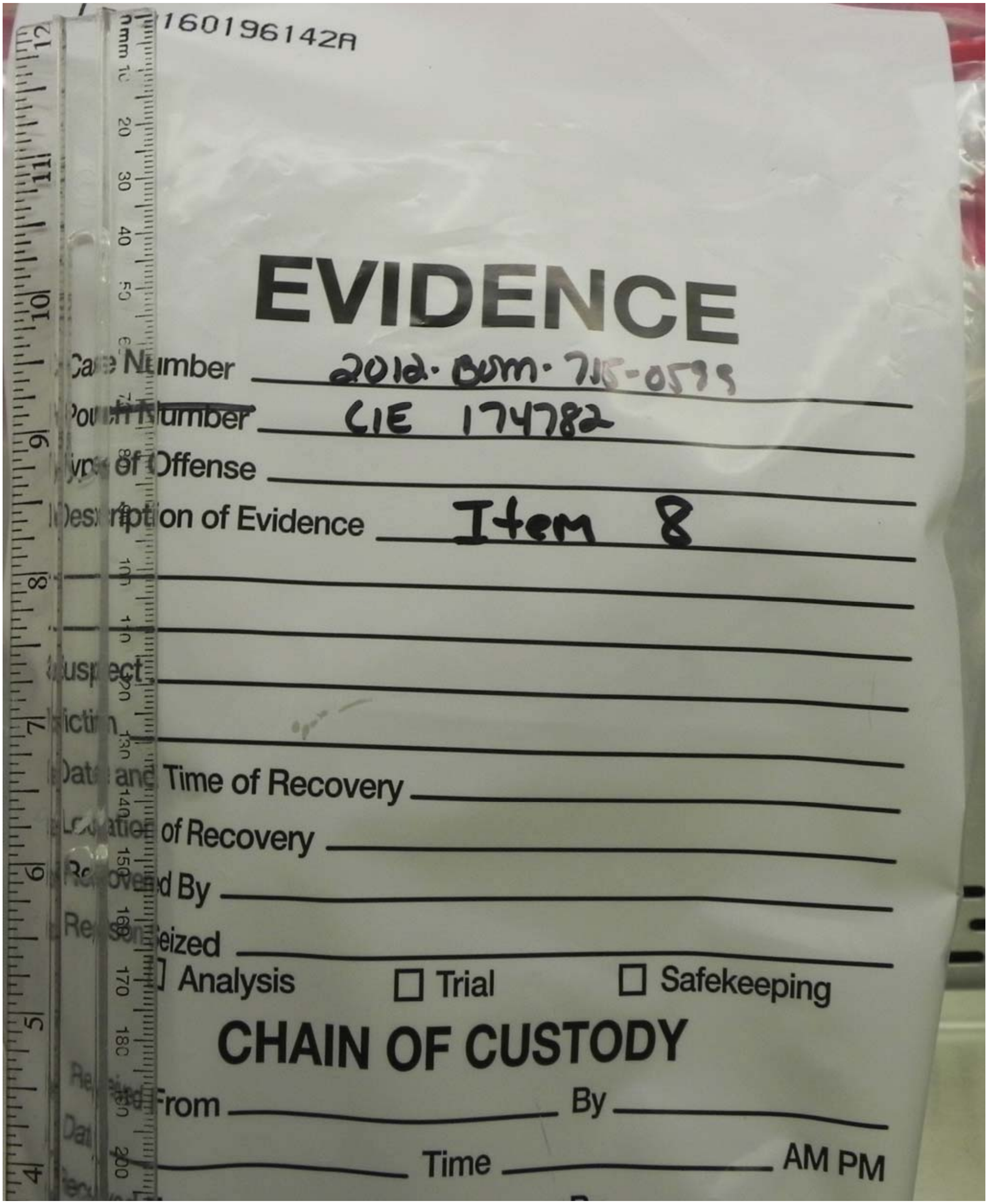


Fig. 12. Representative Item 7 (7A)
Scale in mm



160196142A

EVIDENCE

Case Number 2012·BUM·715-0599

~~Pouch Number~~ CIE 174782

Type of Offense

Description of Evidence Item 8

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized

☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Released From ____ By ____

Date ____ Time ____ AM PM

Fig. 13. Item 8 as received
Scale in mm

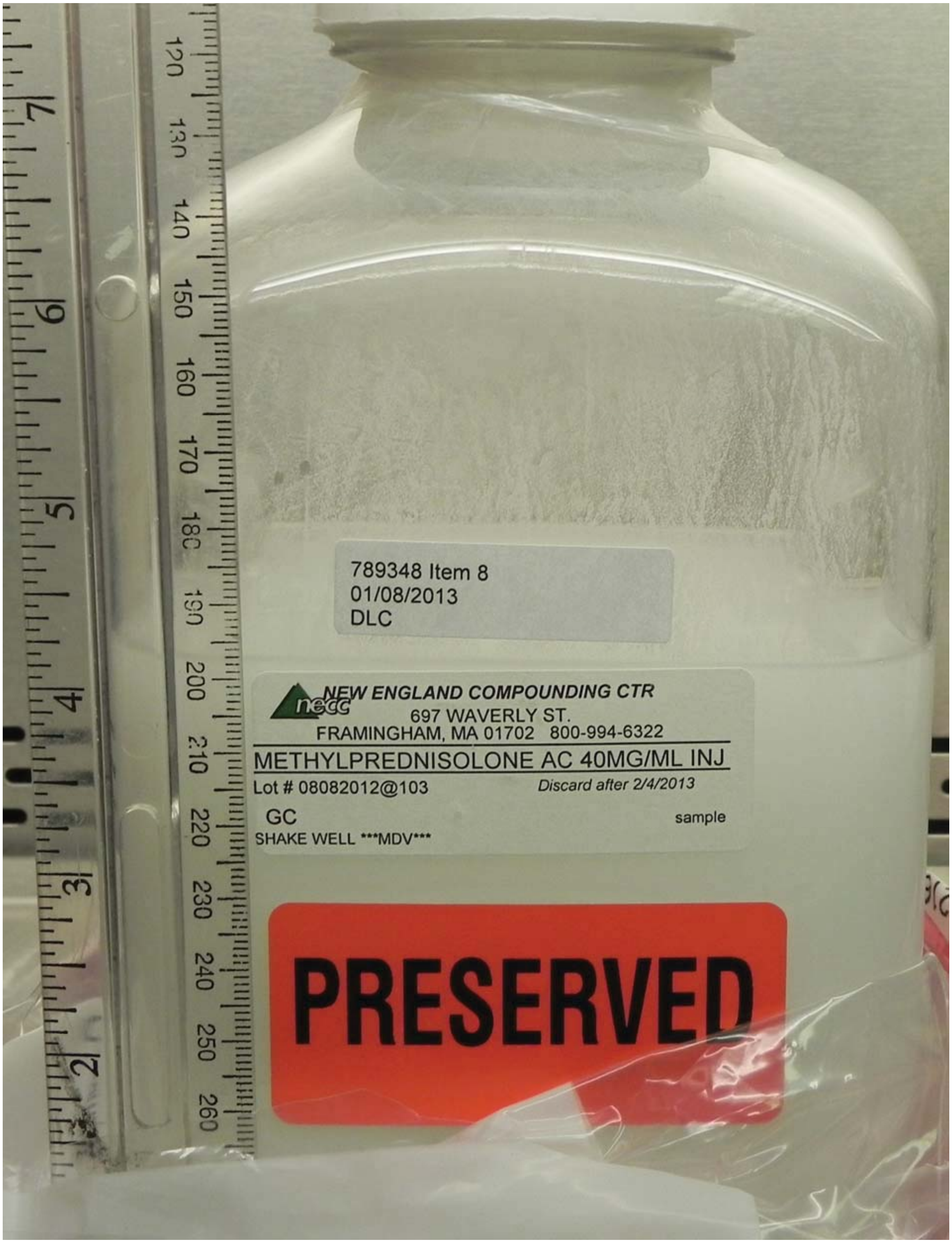
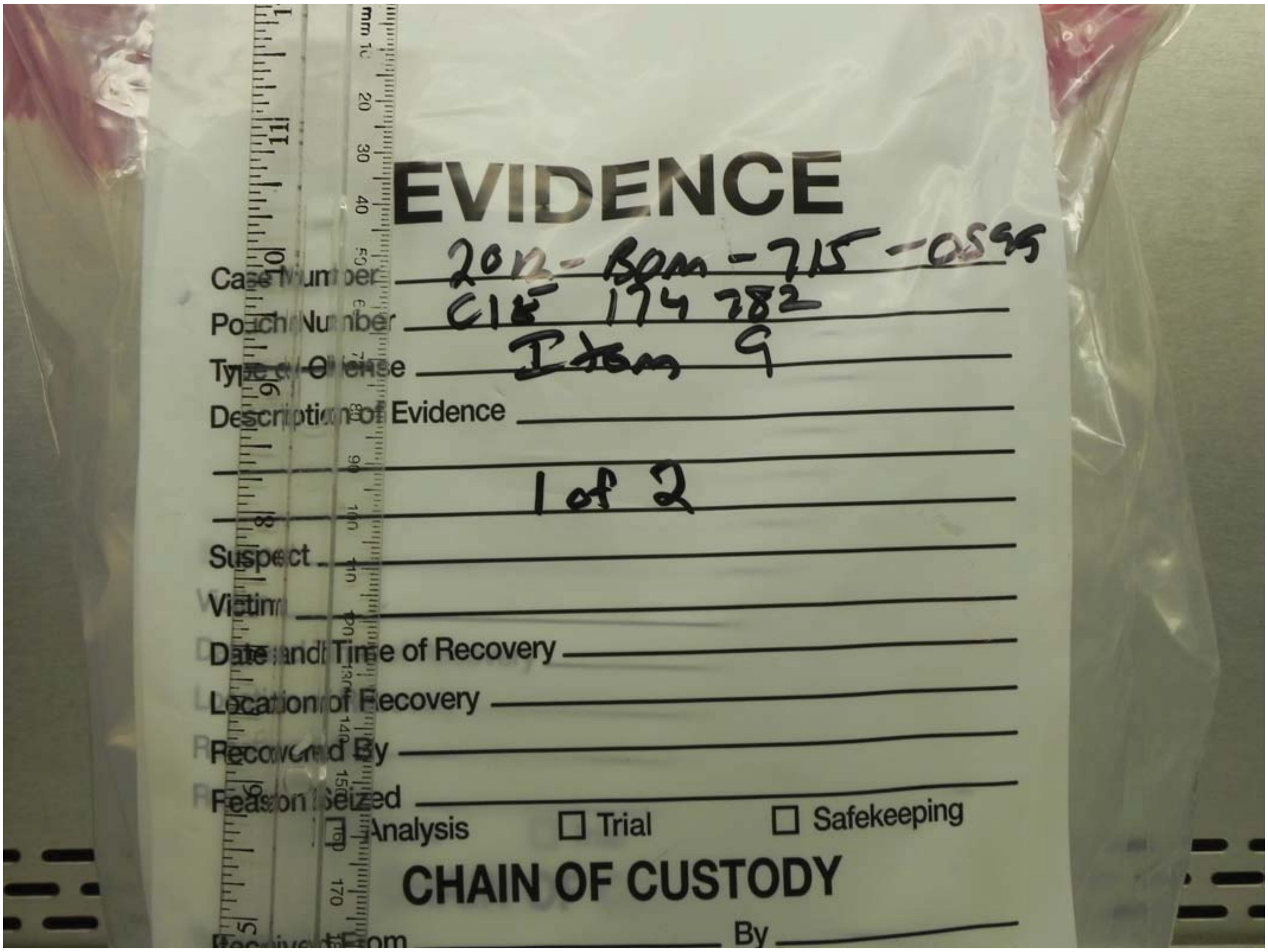


Fig. 14. Item 8
Scale in mm



EVIDENCE

Case Number 2012-Bam-715-0595

Pouch Number C18 174 282

Type of Offense Item 9

Description of Evidence

1 of 2

Suspect

Victim

Date and Time of Recovery

Location of Recovery

Recovered By

Reason Seized ☐ Analysis ☐ Trial ☐ Safekeeping

CHAIN OF CUSTODY

Received From ______ By ______

Fig. 15. Item 9 as received
Scale in mm

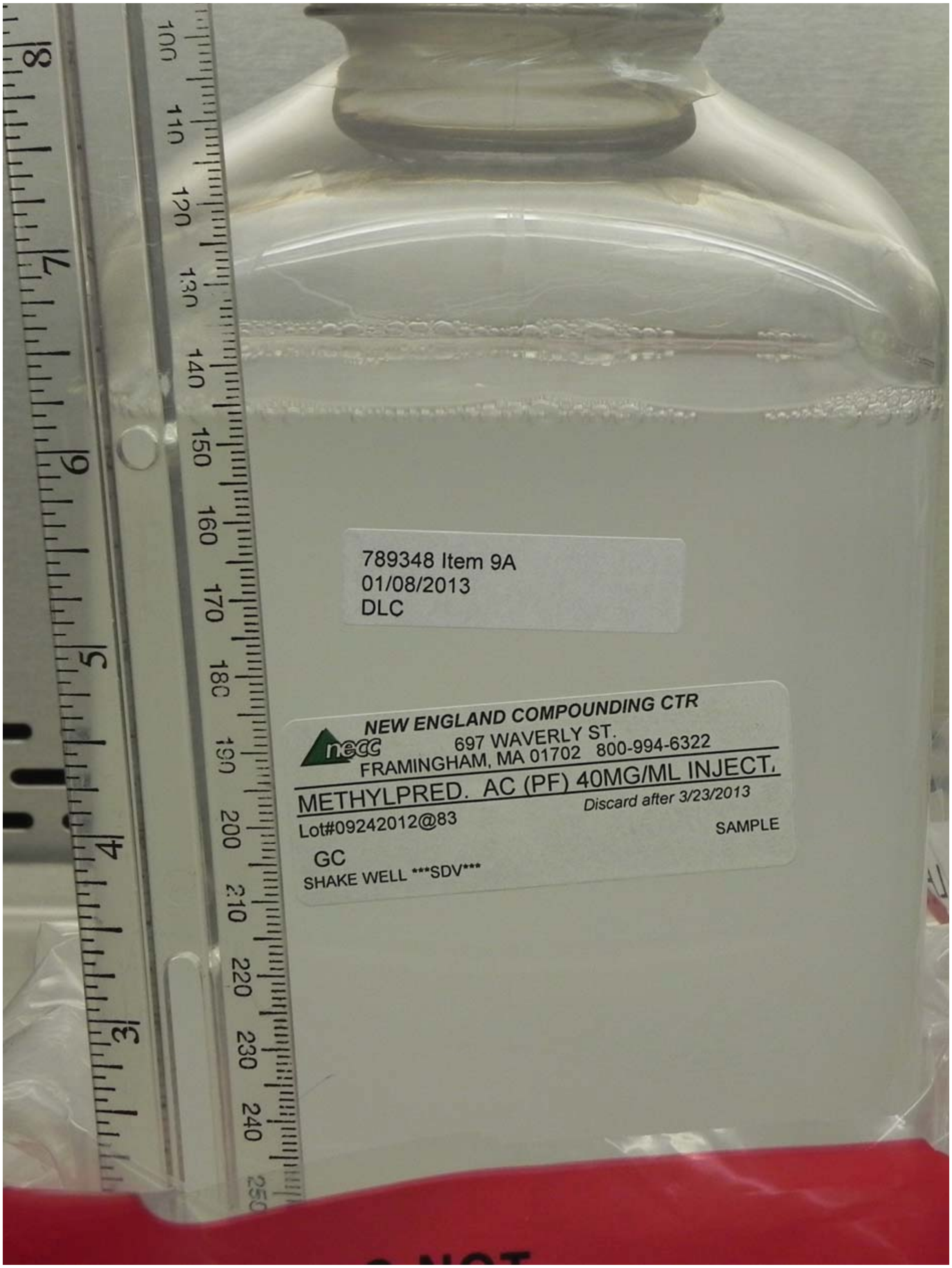


Fig. 16. Representative Item 9 (9A)
Scale in mm